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(54) Title: DIAGNOSTIC PROTEINS TO TEST FOR MORE THAN ONE ANTIBODY

(57) Abstract

This invention is directed to the discovery that certain envelope peptide fragments of the Human Immunodeficiency Virus 2 (HIV-2) are particularly immunoreactive against HIV-2 antibodies. These fragments can therefore be applied to immunodiagnostic tests for the detection of antibodies to HIV-2. This invention is also directed to certain chimeric proteins that are made from immunogenic portions of the envelope gene of HIV-2, HIV-1, or HTLV-I that can be used to test for antibodies to HIV-2, HIV-1, and HTLV-I and HTLV-II.

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DIAGNOSTIC PROTEINS TO TEST FOR MORE THAN ONE ANTIBODY

Field of the Invention

This invention is directed to the discovery that certain envelope peptide fragments of the Human Immunodeficiency Virus 2 (HIV-2) are particularly immunoreactive against HIV-2 antibodies. These fragments can therefore be applied to immunodiagnostic tests for the detection of antibodies to HIV-2. This invention is also directed to certain chimeric peptide fragments that are made from immunogenic portions of the envelope gene of HIV-2, HIV-1, or HTLV-I that can be used to test for antibodies to HIV-2, HIV-1, HTLV-I, and HTLV-II.

BACKGROUND OF THE INVENTION

Human Immunodeficiency Viruses (HIVs) are retroviruses which carry their genetic code on RNA. In humans, Acquired Immune Deficiency Syndrome (AIDS) may be caused by either of two types of HIV: HIV-1 or HIV-2.

HIV-1 (originally HTLV-III) was isolated and identified by Gallo and Montagier et al., U.S. 4,520,113. Ratner et al., Nature 313:277-284 (1985); Muesing et al., Nature 313:450-458 (1985); Sanchez-Pescador et al., Science 227:484-492 (1985); and Wain-Hobson et al., Cell 40:9-17 (1985) disclose the complete DNA sequence of the HTLV-III virus.

HIV-2 (originally LAV-2) was first isolated from West Africans with AIDS-like illness (Clavel, F. et al., Science 233:343-346 (1986); Clavel, F. et al., Nature 324:691-695 (1986)).

HIV-1 and HIV-2 are related to the human T-cell leukemia-lymphoma (HTLV) virus family of T4 tropic retroviruses. HTLV-I is the etiological agent of T-cell leukemia and lymphoma. HTLV-II, originally isolated from a patient with hairy-cell leukemia, is associated with the development of malignant leukemia of mature T-lymphocytes. The complete nucleotide sequence of the provirus genome of HTLV-I is given in Seiki et al., Proc. Nat'l Acad. Sci. (USA) 80:3618-3622 (1983).

When a retrovirus infects a host cell, a DNA copy of its genome is integrated into the chromosome of its host. With some retroviruses, the DNA is integrated into the host cell's chromosomes in the form of a sequence known as a provirus. The DNA copy of the retrovirus' genetic code is synthesized by a viral enzyme called RNA dependent DNA polymerase, or reverse transcriptase. The host cells transcribe the DNA of the viral gene and synthesize the proteins encoded by the virus, which are then assembled into new viruses.

The HIV viruses and HTLV viruses contain at least (i) a gag gene that encodes the internal structural (neurocapsid or core) proteins, (ii) a pol gene that encodes the reverse transcriptase, and (iii) an env gene that encodes the envelope glycoproteins of the virus.

In early studies with HIV-1, Gallo (Gallo, R.C. et al., Science 224:500:503 (1984); Sarnagadharen, M.G. et al., Science 224:506-508 (1985)) and Montagnier (Barre-Sinoussi, F. et al., Science 220:868-871 (1983)), using Western blots, demonstrated that most AIDS patients had antibodies to HIV-1 antigens. From this work, and because it was not widely appreciated that blots could have nonspecific reactions at p17, p24, and other HIV-1 antigens, it was commonly believed

that gag antigens were the appropriate antigens to be used for screening for HIV-1 antibodies. However, not all AIDS sera is reactive in Western blots against gag antigens. The first and most reliable markers of infections are the presence of antibodies to the envelope protein.

Viral lysates of HIV-1 are currently widely used for the detection of antibodies to HIV-1 in human sera. The viral lysate comes from HIV-1 that has been grown in tissue culture and partially purified as the antigen source. The tests are quite sensitive, but suffer from a relatively high rate of false positives. Many scientists have attributed these false positives to cellular proteins contaminating the virus preparations (Honter, J.B. et al., Lancet 1:1222-1223 (1985); Sayers, M.H., Transfusion 26:113-115 (1986)) and the presence of cross-reactive antibodies (Thiry, L. et al., Science 227:1484 (1985); Volsky, D.J., New Engl. J. Med. 315:457-458 (1986)).

The HTLV-I and HTLV-II virus can be readily transferred from the peripheral blood leukocytes of antibody-positive people to leukocytes of antibody-negative people when the two are cultivated together. Popovic et al., Science 219:856-859 (1983). Consequently, there is a risk of infection involved in whole blood transfusions when the transfused blood contains infected cells.

Moreover, individuals infected with one retrovirus may also be harboring a second retrovirus infection. Biological specimens from people with an HIV-1 infection often also give a positive test for antibodies to HTLV-I or HTLV-II. Assays were conducted in the following studies for HTLV-I and HIV-1 antibodies in patient sera: "Rossi et al., Eur. J. Cancer Clin. Oncol 22:411-418 (1986); Aoki et al., Lancet, October 20, 1984, pages 936-937; Tedder et al., Lancet, July 21, 1984, pages 125-128; and Robert-Guroff et al., Lancet, July 21, 1984, pages 128-130. In these studies, the HTLV-I was

detected by viral lysate (Rossi et al. and Aoki et al.) and with core antigen (Tedder et al. and Robert-Guroff et al.).

The Pasteur ELAVIA mixed assay has been reported to preferentially detect HIV-2 antibodies over HIV-1 antibodies. (Lelie, supra). However, this assay is based on the use of a HIV-2 viral lysate assay and thus requires the use of whole virus.

In the United States, human blood must be screened for antibodies to HIV-1 and to HTLV-I and for hepatitis. It is expected that soon human blood sera will also need to be screened for antibodies to HIV-2. Thus, it would be desirable to have a reliable screening assay to test for the presence of antibodies to HIV-2 in human biological samples. It would also be desirable to have a single test that would be able to detect more than one antibody in human biological samples.

SUMMARY OF THE INVENTION

The invention comprises the amino acid sequences of immunoreactive env HIV-2 peptides that can be used for detecting antibodies to HIV-2. The invention also comprises the amino acid sequences of immunodiagnostic chimeric env peptides which are able to detect more than one antibody. Specifically, these chimeric env peptides are able to detect antibodies to HIV-2, HIV-1, and HTLV-I and HTLV-II. Thus, this invention comprises chimeric env peptides comprising immunodiagnostic env peptide fragments of HIV-2-HIV-1; HIV-2-HIV-1-HTLV-I; HIV-2-HTLV-I; and HIV-1-HTLV-I.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the construction of plasmid pK1 from plasmids K3D, containing the HIV-1 clone K3D, and pCBC1.

Figure 2 is an electrophoretic gel showing an induced 34 kd protein, called K1.

Figure 3 is a Western blot showing the reaction of the induced protein, K1, with human sera from HIV-2 infected individuals.

Figure 4 gives the complete DNA sequence of the coding region of the K1 and the derived amino acid sequence.

Figure 5 shows the construction of plasmid pK3 and a 1038 base pair fragment from position 7101 to position 8139 and the ligation of K3D into the expression vector pCBC2.

Figure 6 is an electrophoretic gel showing an induced 40 kd protein, called K3.

Figure 7 is a Western blot showing the reaction of the induced protein, K3, with human sera from HIV-2 infected individuals.

Figure 8 gives the complete DNA sequence of the coding region of the K3 and the derived amino acid sequence.

Figure 9 shows the construction of the plasmid pK1DG71 from plasmids pLCBCODG71A and pK1.

Figure 10 is an electrophoretic gel showing an induced 55 kd chimeric protein, called K1DG71.

Figure 11A is a Western blot showing the reaction of the induced protein, K1DG71, with human sera from HIV-1 infected individuals.

Figure 11B is a Western blot showing the reaction of the induced protein, K1DG71, with human sera from HIV-2 infected individuals.

Figure 12 gives the complete DNA sequence of the coding region of the pK1DG71 and the derived amino acid sequence.

Figure 13 shows the construction of the plasmid pDG71353.

Figure 14 shows the construction of the plasmid pK1DG71353.

Figure 15 gives the complete DNA sequence of the coding region of the pK1DG71353 and the derived amino acid sequence.

Figure 16 is an electrophoretic gel showing an induced 68 kd chimeric protein, K1DG71353.

Figure 17 is a Western blot showing the reaction of the induced protein, K1DG71353, with human sera from HIV-1 positive individuals.

Figure 18 is a Western blot showing the reaction of the induced protein, K1DG71353, with human sera from HIV-2 positive individuals.

Figure 19 is a Western blot showing the reaction of the induced protein, K1DG71353, with human sera from HTLV-I positive individuals.

Figure 20 shows that no specific reactivity was seen with negative sera in a Western blot of the induced protein K1DG71353.

Figure 21 gives the complete DNA sequence of the coding region of the K1DG71353 protein and the derived amino acid sequence.

DEFINITIONS

In the description that follows, a number of terms used in recombinant DNA technology and immunology are extensively utilized. In order to provide a clearer and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

Promoter. A DNA sequence located proximal to the start codon at the 5′ end of the transcribed sequence. At the promoter region, transcription of an adjacent operably linked gene(s) is initiated.

Gene. A DNA sequence that contains information for transcription of a mRNA which codes for a polypeptide or protein. Typically, the nucleotide of the first transcribed codon is numbered +1, and the nucleotides are numbered consecutively with positive integers through the transcribed regions of the gene. Nucleotide +1 may or may not also code for the first translated amino acid. A gene may have regions at the 5′ end and 3′ end which are transcribed but which are not translated. Similarly, the gene may or may not contain intron information which must be spliced out prior to translation of the mRNA. The mRNA may or may not have regions at the 5′ end and/or the 3′ end which are not translated.

The numbering of nucleotides in the promoter and transcriptional regulatory region 5′ to the transcribed region proceeds consecutively with negative integers with the 5′ nucleotide next to the first transcribed codon being numbered -1.

A "complementary DNA" or "cDNA" gene includes recombinant genes synthesized by reverse transcription of mRNA and from which intervening sequences (introns) have been removed.

Operably linked. As used herein, operably linked means that two elements are physically arranged such that factors which influence one element also influence the other. For example, factors which induce a specific promoter to function also induce the transcription of a gene operably linked to that promoter.

Expression. Expression is the process by which the information encoded within a gene is revealed. If the gene encodes a protein, expression involves both transcription of the DNA into mRNA and translation of the mRNA into protein.

Cloning vehicle. A plasmid or phage DNA or other DNA sequence which is able to replicate autonomously in a host cell, and which is characterized by one or a small number of endonuclease recognition sites at which such DNA sequences may be cut in a determinable fashion without loss of an essential biological function of the vehicle, and into which DNA may be spliced in order to bring about its replication and cloning. The cloning vehicle may further contain a marker suitable for use in the identification of cells transformed with the cloning vehicle. Markers, for example, are tetracycline resistance or ampicillin resistance. The word "vector" is sometimes used for "cloning vehicle."

Expression vehicle. A vehicle or vector similar to a cloning vehicle but which is capable of expressing a gene which has been cloned into it, after transformation into a host. The cloned gene is usually placed under the control of (i.e., operably linked to) certain control sequences such as promoter sequences. Expression control sequences will vary depending on whether the vector is designed to express the operably linked gene in a prokaryotic or eukaryotic host and may additionally contain transcriptional elements such as enhancer elements, termination sequences, tissue-specificity elements, and/or translational initiation and termination sites.

Host. Any organism that is the recipient of a replicable expression vehicle.

Peptide fragment. The term "peptide fragment" is meant to include any amino acid sequence which represents a segment of HIV-2, HIV-1, and/or HTLV-I, which is capable of immunologically reacting with the respective antibody and includes naturally-occurring peptide sequences; synthetic, chemically-synthesized peptide sequences; and expressed, genetically engineered peptide sequences.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention comprises the amino acid sequences of immunoreactive env HIV-2 peptides that can be used for detecting antibodies to HIV-2. The invention also comprises genetic sequences coding for the immunoreactive env peptides, expression vehicles containing the genetic sequences, and hosts transformed therewith.

The invention also comprises the amino acid sequences of immunoreactive chimeric env peptides which are able to detect more than one antibody. Specifically, these chimeric env peptides are able to detect antibodies to HIV-2, HIV-1, and HTLV-I and HTLV-II. Thus, this invention comprises chimeric env peptides comprising immunodiagnostic env peptide fragments of HIV-2-HIV-1; HIV-2-HIV-1-HTLV-I; HIV-2-HTLV-I; and HIV-1-HTLV-I. By a chimeric env peptide is meant a peptide wherein said peptide contains an amino acid sequence which encodes domains of different viral peptides. Two peptides are said to be different peptides if they are not covalently joined in their mature form in the native virus. The inventors have discovered that by combining domains of peptides from different retroviruses there is created a chimeric peptide that is able to detect antibodies to HIV-2, HIV-1, and HTLV-I

and HTLV-II, depending on the particular construction of the chimeric peptide.

The chimeric env peptides may contain domains from any HIV-2, HIV-1, and HTLV-I env peptide as long as those domains are capable of being immunoreactive against their respective antibodies or of being combined with another domain in such a way as to maintain, enhance or induce antibody recognition.

A complete HIV-2 nucleotide sequence from HIV-$2_{SL/ISY}$ is described in (Franchini et al., Proc. Natl. Acad. Sci. USA 86:2433-2437 (1989)). The preferred peptide fragments are those described in Examples 1, below, and shown in Figure 4, which gives the complete DNA sequence of the coding region of the K1 peptide fragment and the derived amino acid sequence and in Example 2, below, and shown in Figure 8, which gives the complete DNA sequence of the coding region of the K3 peptide fragment and the derived amino acid sequence.

The preferred env peptides of HIV-1 are those described in Beltz et al., U.S. 4,753,873, particularly the clone G peptide fragment and those peptide fragments derived therefrom. The most preferred env peptide fragment is CBre3, derived from the gp120 and gp41 regions of the HIV-1 env gene. CBre3, also identified as delta G71A, is described in Thorn et al., "An enzyme immunoassay using a novel recombinant polypeptide to detect human immunodeficiency virus," J. Clin. Microbiol. 25:1207-1212 (1987) and in Beltz et al., U.S. 4,753,873. A cell line expressing the recombinant antigen is also on deposit at the American Type Culture Collection (ATCC), accession number 53455. Other recombinant HIV-1 antigens may be used in this invention, provided that the antigens exhibit immunoreactivity to HIV-1 antibodies. Examples of such recombinant HIV-1 antigens are described in Chang, et al., Bio/Technology, 3:905-909 (1985); Cabradilla et

al., Bio/Technology, 4:128-133 (1985); and U.S. 4,629,783, all incorporated herein by reference.

Essex et al., PCT/US84/00561, publication no. WO 84/04327, describes the env glycoproteins of HTLV-I. Any immunoreactive peptide fragment encoded by the env gene of HTLV-I may be used in this invention. The preferred peptide fragment is the HTLV-I envelope gene from base pairs 6101-6118 and 6170-6499 based on the published sequence of Seiki et al. (Proc. Natl. Acad. Sci. USA 80:3618 (1983)) incorporated herein by reference. Other preferred peptide fragments are those described in Samuel et al., Science 126:1094-1097 (1984), incorporated herein by reference, which gives a restriction map of the env gene of HTLV-I. HTLV-II antibodies can also be detected with use of HTLV-I antigens.

As will be understood by one of skill in the art, there may be variations in the first one or two amino acids of the peptide fragments due to proper alignment of the cloned nucleotide sequence in the expression vehicle.

Also as will be understood by one of skill in the art, there may also be some variation in the peptide fragments, provided however, that these peptides retain immunoreactivity to antibodies to HIV-2, HIV-1, or HTLV-I, respectively. Thus the ranges in the length of the peptide fragments need not be precisely fixed. Amino acids of the peptide fragments may be deleted or added without loss of immunoreactivity. Additionally, amino acids could be exchanged, e.g. a neutral amino acids such as a valine could be exchanged with another neutral amino acid such as leucine. The changes in amino acids may be either conservative or non-conservative. There is also genomic variation in amino acid sequence and nucleotide sequence between viral isolates (see, for example, Wong-Staal, F. et al., Science 229:759-762 (1985)). It is to be understood that such variations are included in the peptide fragments of this invention, provided that the peptide

fragment is able to detect the appropriate antibody. By conservative substitutions is intended combinations such as gly, ala; val, ile, leu; asp, glu; asn, gln; ser, thr; lys, arg; and phe, tyr.

In the description of the methods that follow, the HIV-2 env peptide fragments are used for illustration. The HIV-1 and HTLV-I env peptides used in the chimeric env diagnostic peptides may also be used in these described methods.

The native peptide fragments of the HIV-2 virus may be used as a source of the peptide domains and may be obtained directly from the infected host cell. The peptide fragment would then be obtained by fragmenting the naturally-occurring virus using suitable enzymes or chemical methods.

It is also possible to obtain the peptide fragments by chemical synthesis, for example, by well known solid phase peptide synthesis methods (Merrifield, J. Am. Chem. Soc. 85:2149 (1962); Bodanszky, M., Peptide Chemistry: A Practical Textbook, 1988, Springer-Verlag, New York).

A preferred method of obtaining the peptide fragment is by cloning a polynucleotide fragment which codes for the desired peptide, using genetic engineering techniques. The advantages of using genetic engineering and recombinant clones are twofold: the first advantage is that it is difficult and time-consuming to obtain large amounts of the viral peptides either by direct isolation from the virus or by chemical synthesis; the second advantage is that recombinant peptides are devoid of human antigens that may reduce the reliability of a diagnostic test.

The genetic constructs and the methods for using them can be utilized for expression of the peptide fragments in hosts, including prokaryotic and eukaryotic hosts.

In a preferred embodiment, the introduced sequence is incorporated into a plasmid or viral vector capable of autonomous replication in a prokaryotic host. Any of a wide

variety of vectors may be employed for this purpose, as outlined below.

Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species. The procaryotic hosts may include bacteria such as E. coli, S. typhimurium, Serratia marcescens or Bacillus subtilis. Preferably, the peptides of the invention are expressed in prokaryotes and especially in E. coli.

The preferred bacterial host for expression is an E. coli strain that contains a temperature sensitive bacteriophage lambda CI857 gene, such as MZ1, described in Lautenberger et al., Gene Anal. Tech. 1:63-66 (1984). Suitable vector systems for expression in E. coli are pCBC1, described by Beltz, U.S. 4,753,873, and pJL6 Lautenberger, et al., Gene Anal. Tech. 1:63-66 (1984) wherein the bacteriophage lambda pL promoter, synthetic ribosome binding site and the first 13 amino acids from the bacteriophage lambda C_{II} gene are provided on the vector together with a BamHI site for cloning purposes and synthetically derived DNA containing translation termination codons in all three reading frames.

Eukaryotic hosts may also be used and include yeast, filamentous fungi, insect cells and mammalian cells (Smith et al., Mol. Cell Biol. 3:2156-2165 (1983)) and especially mammalian cells which have been immortalized and may be maintained in cell culture.

Preferred eukaryotic plasmids include those derived from the bovine papilloma virus, vaccinia virus, SV40, and, in yeast, plasmids containing the 2-micron circle, etc., or their derivatives. Such plasmids are well known in the art (Bot-

stein, D., et al., Miami Wntr. Symp. 19:265-274 (1982); Broach, J.R., In: The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470 (1981); Broach, J.R., Cell 28:203-204 (1982); Bollon, D.P., et al., J. Clin. Hematol. Oncol. 10:39-48 (1980); Maniatis, T., In: Cell Biology: A Comprehensive Treatise, Vol. 3, Gene Expression, Academic Press, NY, pp. 563-608 (1980)), and are commercially available. For example, mammalian expression vector systems which utilize the MSV-LTR promoter to drive expression of the cloned gene, and in which it is possible to cotransfect with a helper virus to amplify plasmid copy number, and, integrate the plasmid into the chromosomes of host cells have been described (Perkins, A.S. et al., Mol. Cell Biol. 3:1123 (1983); Clontech, Palo Alto, California).

The DNA sequence of the peptide fragments can also be inserted into the genome of viruses which is used to infect a cell; for example, vaccinia virus and bacculovirus may be used. (Mackett, M. et al., Proc. Natl. Acad. Sci. USA 79:7415 (1982); Panicali, D. et al., Proc. Natl. Acad. Sci. USA 80:5364 (1983); and Smith, G.L. et al., Nature 302:490 (1983)) The recombinant vaccinia virus replicates in any mammalian cell and the fragment of interest appears on the envelope or in internal viral proteins.

The DNA sequence may be chemically constructed if it is not desired to utilize the HIV-2 genome as the source of the genetic information. Methods of chemically synthesizing DNA are well known in the art (Oligonucleotide Synthesis, A Practical Approach, M. J. Gait, ed., IRS Press, Washington, D.C., 1984; Synthesis and Applications of DNA and RNA, S.A. Narang, ed., Academic Press, San Diego, CA, 1987). Because the genetic code is degenerate, more than one codon may be used to construct the DNA sequence encoding a particular amino

acid (Watson, J.D., In: Molecular Biology of the Gene, 3rd Ed., W.A. Benjamin, Inc., Menlo Park, CA (1977), pp. 356-357).

To express the recombinant HIV-2 peptides of the invention, transcriptional and translational signals recognizable by an appropriate host are necessary. The cloned HIV-2 peptide encoding sequences, obtained through the methods described above, and preferably in a double-stranded form, may be operably linked to sequences controlling transcriptional expression in an expression vector, and introduced into a host cell, either prokaryote or eukaryote, to produce the recombinant HIV-2 peptides of the invention or functional derivatives thereof.

Expression of the HIV-2 peptides in different hosts may result in different post-translational modifications which may alter the properties of the peptides. It is necessary to express the peptides in a host wherein the ability of the peptides to be immunologically recognized by HIV-2 antibodies is not hindered.

In general, expression vectors containing transcriptional regulatory sequences, such as promoter sequences, which facilitate the efficient transcription of the inserted gene fragment, and which are derived from species compatible with the host cells, are used in connection with these hosts. The expression vector typically contains discrete elements such as, for example, a) either an origin of replication which allows for autonomous replication of the vector or elements which promote insertion of the vector into the host's chromosome, b) a suitable transcriptional promoter to which the sequence of interest can be operably linked, c) a transcriptional terminator sequence if necessary, and d) specific genes which are capable of providing phenotypic selection in transformed cells. The precise nature of the regulatory regions needed for gene expression will vary between species or cell types and there are many appropriate

expression vector systems that are commercially available (For example, through Pharmacia, Boehringer Mannheim or Clontech).

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains expression control sequences which contain transcriptional regulatory information and such sequences are "operably linked" to the nucleotide sequence which encodes the polypeptide.

An operable linkage is a linkage in which a sequence is connected to a regulatory sequence (or sequences) in such a way as to place expression of the sequence under the influence or control of the regulatory sequence. Two DNA sequences (such as HIV-2 peptide encoding sequence and a promoter region sequence linked to the 5′ end of the encoding sequence) are said to be operably linked if induction of promoter function results in the transcription of the peptide encoding mRNA and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the expression regulatory sequences to direct the expression of the HIV-2 peptide, or (3) interfere with the ability of the HIV-2 peptide template to be transcribed by the RNA polymerase. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence.

Expression of the HIV-2 peptides in eukaryotic hosts requires the use of regulatory regions functional in such hosts, and preferably eukaryotic regulatory systems. A wide variety of transcriptional and translational regulatory sequences can be employed, depending upon the nature of the eukaryotic host. The transcriptional and translational regulatory signals can also be derived from the genomic sequences of viruses which infect eukaryotic cells, such as HIV, SIV, adenovirus, bovine papilloma virus, Simian virus,

herpes virus, or the like. Preferably, these regulatory signals are associated with a particular gene which is capable of a high level of expression in the host cell. If desired, a fusion product of the HIV-2 peptides may be constructed. For example, the sequence coding for the HIV-2 peptides may be linked to a signal sequence which will allow secretion of the protein from, or the compartmentalization of the protein in, a particular host. Such signal sequences may be designed with or without specific protease sites such that the signal peptide sequence is amenable to subsequent removal.

Transcriptional initiation regulatory signals can be selected which allow for repression or activation, so that expression of the operably linked genes can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or are subject to chemical regulation, e.g., metabolite.

Where the native expression control sequence signals do not function satisfactorily in the host cell, then sequences functional in the host cell may be substituted.

The vectors of the invention may further comprise other operably linked regulatory elements such as enhancer sequences, or DNA elements which confer species, tissue or cell-type specific expression on an operably linked gene.

Once the vector or DNA sequence containing the construct(s) is prepared for expression, the DNA construct(s) is introduced into an appropriate host cell by any of a variety of suitable means, for example by transformation of bacterial cells. After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene sequence(s) results in the production of the HIV-2 peptide or in the production of a fragment of this peptide. This

expression can take place in a continuous manner in the transformed cells, or in a controlled manner.

If the HIV-2 peptide DNA encoding sequence and an operably linked promoter is introduced into a recipient host cell as a non-replicating DNA (or RNA) molecule, which may either be a linear molecule or, more preferably, a closed covalent circular molecule which is incapable of autonomous replication, the expression of the HIV-2 peptide may occur through the transient expression of the introduced sequence.

Genetically stable transformants may be constructed with vector systems, or transformation systems, whereby the HIV-2 peptide DNA is integrated into the host chromosome. Such integration may occur de novo within the cell or be assisted by transformation with a vector which functionally inserts itself into the host chromosome, for example, with retroviral vectors, transposons or other DNA elements which promote integration of DNA sequences in chromosomes. A vector is employed which is capable of integrating the desired gene sequences into a mammalian host cell chromosome.

Cells which have been transformed with the HIV-2 peptide-containing DNA vectors of the invention are selected by also introducing one or more markers which allow for selection of host cells which contain the vector, for example, the marker may provide biocide resistance, e.g., resistance to antibiotics, or heavy metals, such as copper, or the like.

The transformed host cell can be fermented (if prokaryotic) or cultured (if eukaryotic) according to means known in the art to achieve optimal cell growth, and also to achieve optimal expression of the cloned HIV peptide sequence fragments. As described hereinbelow, high level of HIV-2 peptide expression for the cloned sequences coding for peptide fragments can be achieved according to a preferred procedure of this invention.

After expression of the cloned HIV-2 peptide fragments, the fragments will typically be recovered and purified according to means known in the art. When bacteria are used as the host, high-level expression of the clones usually results in the formation of insoluble inclusion bodies or aggregates. To purify the expressed proteins, the insoluble inclusion bodies must be made soluble. In the preferred embodiment of this invention, the expressed peptide fragments are purified in a process using N-acylation of amino groups, for example, by citraconylation (Marciani, D.J. et al., Protein Purification: Micro to Macro, Alan. R. Liss, Inc., 1987 pp. 443-458.

An alternative to recombinant genetic engineering techniques for producing peptide fragment antigen includes enzyme polymerase directed in vitro transcription and translation systems. An amplification system for producing peptide fragments is described, for example, in U.S. 4,683,202.

The purified immunogenic and diagnostic peptide fragments according to this invention are specifically recognized by antibodies produced in response to the HIV-2 virus. The HIV-2 antibodies in blood or tissue samples can be detected using the peptide fragments in immunoassays wherein the peptides can be utilized in liquid phase or bound to a solid phase carrier. In addition, the peptide fragments can be detectably labeled in various ways for use in immunoassays for virus. The preferred immunoassays for detecting HIV-2 antibodies using the peptide fragments of this invention include radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), or other assays known in the art, such as immunofluorescent assays, chemiluminescent assays, or bioluminescent assays.

Radioactive isotopes which are particularly useful in assays are ^{3}H, ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{36}Cl, ^{57}Co, ^{58}Co, ^{59}Fe, ^{75}Se, and ^{152}Eu.

While radiolabeling represents one embodiment, alternatively, the peptide sequence or antibodies thereto may also be labeled using fluorescent labels, enzyme labels, free radical labels, avidin-biotin labels, or bacteriophage labels, using techniques known to the art (Chard, Laboratory Techniques in Biology, "An Introduction to Radioimmunoassay and Related Techniques," North Holland Publishing Company (1978).

Typical fluorescent labels include fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, and fluorescamine.

Typical chemiluminescent compounds include luminol, isoluminol, aromatic acridinium esters, imidazoles, acridinium salts, and the oxalate esters. Typical bioluminescent compounds include luciferin, luciferase, and aequorin.

Typical enzymes include alkaline phosphatase, β-galactosidase, glucose-6-phosphate dehydrogenase, maleate dehydrogenase, glucose oxidase, and peroxidase.

Two principal types of enzyme assays are enzyme-linked immunosorbent assay (ELISA) and the homogeneous enzyme immunoassay, also known as enzyme-multiplied immunoassay (EMIT) (Syva Corp.). The EMIT system depends on deactivation of the enzyme in the tracer-antibody complex; the activity can thus be measured without the need for a separation step.

The immunoassays within the scope of the present invention include latex agglutination assays, immunometric assays and competitive assays.

Latex agglutination assays have been described in Beltz, G.A. et al., in Molecular Probes: Techniques and Medical Applications, A. Albertini et al., eds., Raven Press, New York, 1989, incorporated herein by reference. In the latex

agglutination assay, a antigen-coated latex particles and serum to be tested are mixed and the results then read. With samples lacking HIV-2 antibodies, the latex particles remain in suspension and retain a smooth, milky appearance. However, if antibodies reactive with the recombinant antigen are present, the latex particles clump into visibly detectable aggregates. There are several ways to read the results of the latex agglutination assay, including visually with slides, see for example, Riggin et al., EPO Published Patent Application No. 289,339, incorporated herein by reference, and with specially made slides which are read either visually or with a spectrophotometer, for example those devices described in U.S. 4,596,695 and 4,775,515, incorporated herein by reference. The latex agglutination assay is especially suitable for small volume users, emergency situations, and areas lacking the sophisticated laboratory equipment and supplies needed for immunometric assays.

An agglutination assay can also be used to detect HIV-2 antibodies wherein the desired peptide fragment is immobilized on a suitable particle other than latex beads, for example, on gelatin, red blood cells, nylon, liposomes, gold particles, etc. The presence of antibodies in the same causes agglutination, similar to that of a precipitation reaction, which can then be detected by such techniques as nephelometry, turbidity, infrared spectrometry, visual inspection, colorimetry, and the like.

Immunometric assays include forward sandwich, reverse sandwich immunoassays and simultaneous assay. Each of these terms is well understood by those skilled in the art. The immunometric assays will be described for the detection of antibodies to HIV-2. In these assays, the peptide fragment is bound to the solid-phase carrier and anti-IgG antibodies are detectably labeled.

In a forward sandwich immunoassay, a sample suspected of containing antibodies against HIV-2 is first incubated with a solid-phase immunoabsorbent containing the peptide fragment. Incubation is continued for a period of time sufficient to allow the antibodies in the sample to bind to the immobilized peptide fragment. After the first incubation, the solid-phase immunoabsorbent is separated from the incubation mixture and washed to remove interfering substances which also may be present in the sample. Solid-phase immunoabsorbent-containing antibodies bound to the immobilized peptide fragments are subsequently incubated for a second time with soluble labeled antibody cross-reactive with a different domain on the antibody to be detected. After the second incubation, another wash is performed to remove unbound labeled antibody from the solid-phase immunoabsorbent and to remove non-specifically bound labeled antibody. Labeled antibody bound to the solid-phase immunoabsorbent is then detected and the amount of labeled antibody detected serves as a direct measure of the amount of antibodies present in the original sample. Alternatively, labeled antibody which is not associated with the immunoabsorbent complex can also be detected, in which case the measure is in inverse proportion to the amount of antigen present in the sample. Forward sandwich assays are described, for example, in United States Patents 3,867,517; 4,012,294; and 4,376,110.

In a reverse sandwich assay, the sample suspected of containing test antibodies against HIV-2 is initially incubated with labeled anti-antibody, after which the solid-phase immunoabsorbent containing immobilized peptide fragment cross-reactive with a different domain on the test antibody is added thereto, and a second incubation is carried out. The initial washing step required by a forward sandwich assay is not required, although a wash is performed after the second

incubation. Reverse sandwich assays have been described, for example, in U.S. Patent No. 4,098,876 and 4,376,110.

In a simultaneous sandwich assay, the sample, the immunoabsorbent having immobilized peptide fragment thereon and labeled soluble antibody specific to a different domain of the test antibody are incubated simultaneously in one incubation step. The simultaneous assay requires only a single incubation and does not require washing steps. The use of a simultaneous assay is a very helpful technique, providing ease of handling, homogeneity, reproducibility, linearity of the assays, and high precision. A simultaneous sandwich assay is described, for example, in U.S. Patent No. 4,376,110.

So-called delayed immunometric assays can also be utilized, as are, for example, described in Chu, U.S. Patent No. 4,289,747, and Wolters, U.S. Patent No. 4,343,896.

Another immunometric assay involves capturing the Fc capture technique . In the Fc capture immunoassay, total serum antibodies are captured through anti-human Fc antibodies, typically bound to a solid support. Thus, bound, the Fc region of the antibody to be detected does not participate in other protein-protein interactions. The antibodies to be detected can then be screened with the HIV-2 peptide fragment(s) or with the appropriate chimeric peptide fragment(s) of this invention. A preferred Fc capture immunoassay is described in U.S. Serial No. 07/203,730, filed June 8, 1988, incorporated herein by reference.

In each of the above assays, the sample-containing antibody, solid-phase immunoabsorbent with immobilized peptide fragment and labeled soluble antibody are incubated under conditions and for a period of time sufficient to allow the test antibodies to bind to the immobilized peptide fragments and to the soluble antibodies. In general, it is desirable to provide incubation conditions sufficient to bind as much

antibody as possible, since this maximizes the binding of labeled antibody to the solid phase, thereby increasing the signal. Of course, the specific concentrations of labeled antibodies and immobilized fragments, the temperature and time of incubation, as well as other such assay conditions, can be varied, depending upon various factors including the concentration of antibody in the sample, the nature of the sample, and the like. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

There are many solid-phase immunoabsorbents which have been employed and which can be used in the present invention. Well-known immunoabsorbents include beads formed from glass, polystyrene, paper, polypropylene, dextran, nylon, and other material; tubes formed from or coated with such materials, and the like. The immobilized peptide fragments may be covalently or physically bound to the solid-phase immunoabsorbent, by techniques such as covalent bonding via an amide or ester linkage or by adsorption. Those skilled in the art will know many other suitable carriers for binding peptide fragments, or will be able to ascertain such, using routine experimentation.

General competitive binding assay techniques useful for the detection of minute amounts of organic molecules such as hormones, proteins, antibodies, and the like are well-known in the art. See Chard, supra. Any of these competitive binding assay techniques can be used for the purposes of detecting HIV-2 antibodies. In order to carry out a competitive binding assay, typically a radioimmunoassay (RIA), it is necessary to provide a binding molecule which has affinity for the label-containing antibody raised in response to a peptide fragment, and for the HIV-2 antibody to be tested as well. A small amount of the fluid or tissue sample containing an unknown quantity of HIV-2 antibody is incubated in the presence of the

raised labeled antibody and also a known amount of antibody-specific peptide fragment.

The raised antibody is preferably generated with antigenic peptide fragments of the invention. Once the incubation of the test sample with the fragment and tracer-containing antibody is complete, it is necessary to determine the distribution of the tracer-containing molecule between the free and bound (immunocomplexed) form. Usually, but not always, this requires that the bound fraction be physically separated from the free fraction. For example, the specific peptide fragment can be bound to a plate. A variety of other techniques may be used for that purpose, each exploiting physical-chemical differences between the tracer-containing molecule in its free and bound form. The generally available methodologies have been described by Yalow, in Pharmacol. Rev. 28:161 (1973). These techniques include adsorption, precipitation, salting out techniques, organic solvents, electrophoretic separation, and the like. See Chard, supra, pp. 405-422.

As in the immunometric assays described above, the soluble antibody may be labeled with any detectable label, such as a radiolabel, a fluorescent label, an enzyme label, a free radical label, or a bacteriophage label. Most commonly, the label is a radiolabel or an enzyme label.

The HIV-2 immunogenic peptide fragments according to this invention may be used to stimulate the production of antibodies. In order to stimulate the production of antibody, the peptide fragment may be coupled to a carrier protein such as bovine serum albumin or keyhole limpet hemocyanin (KLH), utilizing techniques well-known and commonly used in the art. Preferably, the carrier protein is KLH, linked to the peptide fragment through a cysteine residue.

Additionally, the HIV-2 peptide fragments can be admixed with an immunologically inert or active carrier. Carriers

which promote or induce immune responses, such as Freund's complete adjuvant, can be utilized.

The preparation of antisera in animals is a well-known technique (see, for example, Chard, supra, pp. 385-396; and Antibodies, A Practical Handbook, Vols. I and II, D. Catty, ed., IRL Press, Washington, D.C. (1988)). The choice of animal is usually determined by a balance between the facilities available and the likely requirements in terms of volume of the resultant antiserum. A large species such a goat, donkey and horse may be preferred, because of the larger volumes of serum readily obtained. However, it is also possible to use smaller species such as rabbit or guinea pig which often yield higher titer antisera. Usually, subcutaneous injection of the antigenic material (the peptide fragment hapten-carrier protein conjugate) are introduced into the immune system of the animal in which antibodies are to be raised. The detection of appropriate antibodies may be carried out by testing the antisera with appropriately labeled tracer-containing molecules. Fractions that bind tracer-containing molecules are then isolated and further purified if necessary.

Antibodies thus obtained may then be utilized in various immunoassays to identify and quantitate the HIV-2 virus or fragments thereof. Both polyclonal antibodies and monoclonal antibodies, produced by well-known techniques as described in Catty, supra, raised in response to the peptide fragments of this invention can be utilized in immunoassays.

When one uses immunometric assays to detect the HIV-2 virus or portions thereof, two separate and distinct antibodies are required. One of these antibodies is bound to the solid-phase support while the other is detectably labeled. In essence, the two different antibodies, although specific for HIV-2 virus, are cross-reactive with different domains on viral protein. In one embodiment, the two different

antibodies may be prepared by using two different peptide fragments according to this invention. The use of antibodies to different peptide fragments, one bound to a carrier and the other detectably labeled, is useful in various sandwich assays.

Alternatively, it is also possible to prepare antibodies which are specific to HIV-2 virus, but cross-reactive with different domains by producing the antisera in two different species, for example, in rabbit and in mouse, utilizing the same peptide fragment.

In addition, the materials for use in the assays of the invention are ideally suited for preparation of a kit. Such a kit may comprise a carrier means being compartmentalized to receive in close confinement one or more container means such as vials, test tubes, and the like. Each of said container means comprises one of the separate elements to be used in the method.

For example, one of said container means may comprise an immunoabsorbent-bound peptide fragment. Such fragment may be bound to a separate solid-phase immunoabsorbent or directly to the inner walls of a container. A second container may comprise detectably labeled anti-antibody in lyophilized form or in solution.

The carrier may also contain, in addition, a plurality of containers each of which comprises different, predetermined and known amounts of antibody. These latter containers can then be used to prepare a standard curve from which can be interpolated the results obtained from the sample containing the unknown amount of antibody.

In the practice of this invention, the presence of the HIV-2 antibody or the virus itself or portions thereof may be detected in biological fluids and tissues. Any sample containing the unknown amount of HIV-2 antibodies or HIV-2 can be used. Normally, a sample is a liquid such as, for example,

urine, saliva, tear drops, cerebrospinal fluid, blood, serum and the like, or a solid or semi-solid such as, for example, tissues, feces, and the like. As is known in the art, the HIV-2 virus and antibodies to the virus are associated with the T-cell disorder, Acquired Immune Deficiency Syndrome (AIDS) and pre-AIDS conditions, such as AIDS-related complex (ARC). In addition, it is also known in the art that antibodies to HIV-2 may be present in a human's or animal's biological fluids or tissue, without such human or animal suffering from AIDS or ARC.

The peptide fragments according to this invention may also be used as a vaccine against the HIV-2 virus. The peptide fragment may be prepared and administered to an animal, as is generally known in the art, to stimulate the production of antibodies. Preferably, the vaccinia virus can be used according to known means for the preparation of HIV-2 vaccines.

The following examples further describe the materials and methods used in carrying out the invention. The examples are not intended to limit the invention in any manner.

EXAMPLES

Example 1: Construction of HIV-2 envelope expressing clone MZ-1/pK1

The K3D clone is a subgenomic fragment of a complete HIV-2 clone HIV-$2_{SL/ISY}$ (Franchini et al., Proc. Natl. Acad. Sci. USA 86:2433-2437 (1989)), prepared by insertion of a KPNI fragment of HIV-$2_{SBL/ISY}$ from position 5295 to position 9012 into a Bluescript vector (Stratagene, Inc.). The HIV-2 clone K3D was provided by Genoveffa Franchini.

The strategy for construction of pK1, a plasmid for expression of a peptide fragment of the env region of HIV-2 in E. coli, is illustrated in Figure 1.

K3D DNA was digested with restriction enzyme EcoRI with cuts at position 7297. The EcoRI end was made blunt with Klenow polymerase and BglII linkers were added. The DNA was then digested with BglII and Sau3AI. A fragment of approximately 848 base pairs extending from the EcoRI site (converted to BglII) at position 7297 to the Sau3AI site at position 8139 was purified and cloned into Bam HI restricted the expression vector pCBC1 to generate the clone pK1.

Plasmid pCBC1 is an E. coli expression vector that makes use of the bacteriophage lambda pL promoter. pCBC1 is similar to pJL6 (Lautenberger et al., Gene Anal. Tech. 1:63 (1984)) whose construction is described completely in Beltz et al., U.S. Patent No. 4,753,873, incorporated herein by reference.

Using E. coli strain MZ-1, a strain lysogenic for bacteriophage lambda, C_{I857}, as a host strain for pK1, recombinant protein synthesis was induced by temperature shift from 32°C to 42°C (Naghi et al., Nature 309:810 (1984)). As shown in Figure 2, the temperature shift resulted in the synthesis of a 34 kd protein. Following Western blot transfer of the separated proteins, the induced protein, called K1, reacted specifically with human sera from HIV-2 infected individuals (Figure 3). The complete DNA sequence of the coding region of K1 and the derived amino acid sequence is presented in Figure 4.

Example 2: Construction of HIV-2 envelope expressing clone MZ-1/pK3

K3D (described in Example 1) was digested with Sau3AI. This cutting resulted in a 1038 base pair fragment from position 7101 to position 8139 (Figure 5). After purifying

the fragment, it was ligated into the expression vector pCBC2. pCBC2 differs from pCBC1 by a single base pair just 5′ to the BamHI cloning site. pCBC2 was used in this case rather than pCBC1 to allow for proper reading frame alignment. pCBC2 is also described in Beltz et al., U.S. Patent No. 4,753,873, incorporated herein by reference. The resulting plasmid, pK3, was transferred into the E. coli host MZ-1. Upon temperature shift of MZ-1/pK3 culture from 32°C to 42°C, synthesis of a recombinant protein of 40 kd was induced (Figure 6). Western blot analysis has shown that this protein, called K3, reacted specifically with sera from HIV-2 infected individuals (Figure 7). The complete DNA sequence of the coding region of pK3 and the derived amino acid sequence is presented in Figure 8.

Example 3: Construction of an HIV-2-HIV-1 chimeric envelope expressing clone MZ-1/pK1DG71

The strategy for the construction of the plasmid to express a chimeric envelope clone is illustrated in Figure 9. The plasmid clone pLCBCODG71A has been described in Beltz et al., U.S. 4,753,873, incorporated herein by reference. This plasmid is used for expression of HIV-1 envelope polypeptide. pLCBCODG71A was digested with BglII and BamHI. BglII cleaves at position 407 and BamHI cleaves at position 944. A 537 BP fragment was isolated.

pK1 plasmid DNA, described in Example 1, was digested with BamHI which cleaves at position 890. The 537 BP BglII-BamHI fragment of pLCBCODG71A was ligated to BamHI digested pK1. The resulting plasmid, pK1DG71, was transferred to E. coli host MZ-1. Upon temperature shift of MZ-1/pK1DG71 culture from 32°C to 42°C, synthesis of a recombinant protein of approximately 55 kd was induced (Figure 10). Western blot analysis was done with sera from HIV-I infected individuals,

HIV-2 infected individuals and negative controls. The Western blots (Figure 11) showed that the chimeric HIV-2-HIV-1 protein, K1DG71, reacts with sera from HIV-1 infected individuals (Figure 11A) and sera from HIV-2 infected individuals (Figure 11B). No reactivity was seen with negative sera (Figure 11C).

The complete DNA sequence of the coding region of pK1DG71 and the derived amino acid sequence is presented in Figure 12.

Example 4: Construction of an HIV-2-HIV-1-HTLV-I-Chimeric Envelope Expressing Clone

The strategy for construction of an HIV-2-HIV-1-HTLV-I chimeric envelope expressing clone is illustrated in Figure 13 and Figure 14. pCB1353 is an expression plasmid containing regions of the HTLV-I envelope gene from base pairs 6101-6118 and 6170-6499 based on the published sequence of Seiki et al. (Proc. Natl. Acad. Sci. USA 80:3618 (1983)) cloned into expression plasmid pCBC1. The plasmid pCBC1353 was digested with BamHI and SalI to generate a 370 base pair fragment. The 370 BP fragment was inserted into the plasmid pCB2DG71 which had been digested with BamHI and SalI. pCBC2DG71 is an expression plasmid coding for a polypeptide of the HIV-1 envelope protein. This plasmid has been described by Beltz et al., U.S. 4,753,873, incorporated herein by reference. The resulting plasmid, pDG71353, codes for a chimeric polypeptide of HIV-1-HTLV-I. The sequence of the coding region of pDG71353 is shown in Figure 15.

Figure 14 shows the remaining steps for the HIV-2-HIV-1-HTLV-I construction. pDG71353 was digested with HindIII and SalI. The insert which contains sequences coding for the HIV-1-HTLV-I polypeptide was ligated into the vector pK1DG71 which had been digested with HindIII and SalI. pK1DG71 is described

in Example 3. The resulting plasmid is called pK1DG71353. pK1DG71353 was transferred into the bacterial strain MZ-1.

When MZ-1 pK1DG71353 was induced by a temperature shift from 32°C to 42°C, a protein of 68 kd was produced (Figure 16). Following Western blot transfer of the separated protein, the induced protein reacted specifically with sera from HIV-1 positive individuals, Figure 17; with sera from HIV-2 positive individuals, Figure 18; and with sera from HTLV-I positive individuals, Figure 19. No specific reactivity was seen with negative sera (Figure 20). The complete sequence of the coding region of pK1DG71353 is shown in Figure 21.

Example 5: Purification of HIV-2 env (K-1) and HIV-2-HIV-1 Chimeric Antigens

The following purification procedure has been used to purify both HIV-2 env (K-1) and HIV-2-HIV-1 chimeric antigens.

E. coli cells were lysed by enzymatic digestion with lysozyme (1 mg/gram of cells) for 10 minutes in 50 mM TrisHCl, pH 7.5 (3 ml/gram of cells) containing 2 mM PMSF, aprotinin (0.1 mg/gram of cells) and DNase I (0.1 mg/gram of cells). The solution was brought up to 1% triton X-100 and stirred for 30 minutes at room temperature. Insoluble material was collected by centrifugation at 12,000 xg for 30 minutes and redigested as above with the exception that RNase I (0.05 mg/gram of cells) and 2.5 mM $MgCl_2$ were added. After digestion, the pellet was collected by centrifugation as above.

The insoluble material was then sequentially washed with the following buffers (0.2 gram of cells/ml):

1) 50 mM TrisHCl, pH 9.0 containing 10 mM EDTA and 0.5% Zwittergent 3-14,
2) 50 mM TrisHCl, pH 9.0/1M NaCl,

3) 6M urea in 50 mM TrisHCl, pH 7.5,

4) 8M urea in 50 mM TrisHCl, pH 9 with 1% β-mercaptoethanol.

In each case, the recombinant antigens remained in the insoluble pellet. The pellet from the 8M urea wash was then solubilized with 7M guanidine-HCl pH 11.0/0.5% β-mercaptoethanol. Both HIV-2 env antigen and HIV-2-HIV-1 chimeric protein are very hydrophobic and tend to form aggregates upon removal of guanidine-HCl. To prevent this aggregation, the solubilized antigens were subjected to the following chemical modifications designed to increase the solubility of the antigens in aqueous buffers.

The solubilized protein in 6M guanidine-HCl was first alkylated with 1.2 fold excess of iodoacetic acid over β-mercaptoethanol at pH 8.5. Any unreacted iodoacetic acid was quenched with β-mercaptoethanol. The alkylated sample was dialyzed 3 times against 200 fold 50 mM borate, pH 9.0. The protein became insoluble upon dialysis. The insoluble material was collected by centrifugation and redissolved in 8M Urea, 50 mM borate, pH 9.0 for acylation.

To acylate the alkylated antigen, a 50-fold excess of citraconic anhydride over amino groups on the antigen was added and the pH of the solution was maintained between 8.5 to 9.0 with NaOH. After the reaction was complete, the citraconylated sample was dialyzed against 50 mM borate, pH 9.0 at 4°C. After dialysis, the citraconylated protein was soluble in aqueous buffer and amenable to standard chromatographic technique.

The modified protein sample was then applied to a DEAE-TSK column, equilibrated in 50 mM borate, pH 9.0. The column was developed with a linear gradient of 0.3-1M NaCl to elute the recombinant antigen. Fractions containing HIV-2 env, K-1, antigen or HIV-2-HIV-1 chimeric protein, K1DG71, were pooled.

The immunoreactivity of purified K1 and the chimeric K1DG71 antigens were tested using an indirect immunoassay (EIA). Recombinant antigen was coated onto microtiter wells at concentrations of 0.5 ug and 1.0 ug for K-2 and the chimeric antigen respectively. After washing and blocking non-specific binding sites on the wells, human sera diluted 1:20 was added and any antibodies present allowed to bind to the recombinant antigen for 1 hour at 37°C. Wells were washed to remove unbound antibodies and the presence of bound antibody was detected with horse radish peroxidase labelled goat anti human antibody and a substrate of TmB (3,3', 5,5'-tetramethy/benzidine). Reactions were terminated with H_2SO_4 and OD_{490} determined. Distribution of signals obtained with each antigen for various sera are presented in Table 1.

Table 1

Number of Samples in each OD_{490} Range

Sample	Antigen	<0.2	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	1.0-1.2	1.2-1.4	1.4-1.6	1.6-1.8	1.8-2.0	>2.0
HIV-2 Pos	K1	0	0	0	1	0	0	1	7	24	29	12
HIV-1, HIV-2 Neg	K1	278	1	0	0	0	0	0	0	0	0	0
HIV-1 Pos	Chimeric	0	0	0	1	0	0	0	0	1	1	109
HIV-2 Pos	Chimeric	0	0	0	0	1	0	0	0	1	1	71
HIV-1, HIV-2 Neg	Chimeric	275	5	0	0	0	0	0	0	0	0	0

A101-03.TAB

Now having fully described this invention, it will be understood by those with skill in the art that the scope may be performed within a wide and equivalent range of condition, parameters, and the like, without affecting the spirit or scope of the invention or of any embodiment thereof.

WHAT IS CLAIMED IS:

1. A peptide fragment encoded by nucleotides of about 848 base pairs of the envelope region of the HIV-2 virus wherein said peptide fragment is encoded by the HIV-2 provirus nucleotides at position 7297 to position 8139 as shown in Figure 4.

2. A peptide fragment encoded by nucleotides of about 1038 base pairs of the envelope region of the HIV-2 virus wherein said peptide fragment is encoded by the HIV-2 provirus nucleotides at position 7101 to position 8139 as shown in Figure 8.

3. A chimeric HIV-2-HIV-1 peptide fragment comprising an immunodiagnostic peptide fragment the envelope region of the HIV-2 virus and an immunodiagnostic peptide fragment of the envelope region of the HIV-1 virus.

4. A chimeric HIV-2-HIV-1 peptide fragment comprising said immunodiagnostic peptide fragment of the envelope region of the HIV-2 virus of claim 1 and an immunodiagnostic peptide fragment of the envelope region of the HIV-1 virus.

5. The chimeric HIV-2-HIV-1 peptide fragment of claim 4 wherein said immunodiagnostic peptide fragment of the envelope region of the HIV-1 virus is selected from the group consisting of clone G or peptide fragments derived therefrom.

6. The chimeric HIV-2-HIV-1 peptide fragment of claim 4 wherein said immunodiagnostic peptide fragment of the envelope region of the HIV-1 virus is CBre3.

7. A chimeric HIV-2-HIV-1-HTLV-I peptide fragment comprising an immunodiagnostic peptide fragment the envelope region of the HIV-2 virus, an immunodiagnostic peptide fragment of the envelope region of the HIV-1 virus, and an immunodiagnostic peptide fragment of the envelope region of the HTLV-I virus.

8. The chimeric HIV-2-HIV-1-HTLV-I peptide fragment of claim 7 wherein said immunodiagnostic peptide fragment of the envelope region of the HIV-2 virus is encoded by the HIV-2 provirus nucleotides at position 7297 to position 8139 as shown in Figure 4 or is encoded by the HIV-2 provirus nucleotides at position 7101 to position 8139 as shown in Figure 8.

9. The chimeric HIV-2-HIV-1-HTLV-I peptide fragment of claim 7 wherein said immunodiagnostic peptide fragment of the envelope region of the HIV-1 virus comprises the HIV-1 coding sequence of K1DG71353 as shown in Figure 21.

10. A chimeric HIV-2-HTLV-I peptide fragment comprising an immunodiagnostic peptide fragment the envelope region of the HIV-2 virus and an immunodiagnostic peptide fragment of the envelope region of the HTLV-I virus.

11. The chimeric HIV-2-HTLV-I peptide fragment of claim 10 wherein said immunodiagnostic peptide fragment of the envelope region of the HIV-2 virus is encoded by the HIV-2 provirus nucleotides at position 7297 to position 8139 as shown in Figure 4 or is encoded by the HIV-2 provirus nucleotides at position 7101 to position 8139 as shown in Figure 8.

12. The chimeric HIV-2-HTLV-I peptide fragment of claim 10 wherein said immunodiagnostic peptide fragment of the envelope region of the HTLV-I virus is encoded by the HTLV-I provirus nucleotides at position 6101-6118 and 6170-6499.

13. A chimeric HIV-1-HTLV-I peptide fragment comprising an immunodiagnostic peptide fragment the envelope region of the HIV-1 virus and an immunodiagnostic peptide fragment of the envelope region of the HTLV-I virus.

14. The chimeric HIV-1-HTLV-I peptide fragment of claim 13 wherein said immunodiagnostic peptide fragment of the envelope region of the HIV-1 virus is selected from the group consisting of clone G or peptide fragments derived therefrom.

15. The chimeric HIV-1-HTLV-I peptide fragment of claim 13 wherein said immunodiagnostic peptide fragment of the envelope region of the HIV-1 virus is CBre3.

16. The chimeric HIV-1-HTLV-I peptide fragment of claim 13 wherein said immunodiagnostic peptide fragment of the envelope region of the HTLV-I virus is encoded by the HTLV-I provirus nucleotides at position 6101-6118 and 6170-6499.

17. A recombinant vector for transforming a host cell comprising a DNA encoding any of the peptide fragments of claims 1 to 16 or 22-24.

18. A method for detecting HIV-2 antibodies comprising the steps of:

(a) contacting a sample suspected of containing antibodies to HIV-2 with a peptide fragment of any of claims 1-12 or 22-24 and

(b) detecting the presence of said antibodies.

19. A method for detecting HIV-1 antibodies comprising the steps of:

(a) contacting a sample suspected of containing antibodies to HIV-2 with a peptide fragment of any of claims 3-9 or 22-24 and

(b) detecting the presence of said antibodies.

20. A method for detecting HTLV-I and/or HTLV-II antibodies comprising the steps of:

(a) contacting a sample suspected of containing antibodies to HIV-2 with a peptide fragment of any of claims 7-16 and

(b) detecting the presence of said antibodies.

21. A kit for detecting either HIV-2, HIV-1 or HTLV-I and HTLV-II antibodies, or a combination of antibodies thereof, in a sample comprising a carrier being compartmentalized to receive one or more containers in close confinement therein and further comprising

1. a first container means comprising a peptide fragment of any of claims 1-16 or 22-24; and

2. a detection system for determining the presence of HIV-2, HIV-1, or HTLV-I and HTLV-II antibodies, or a combination thereof.

22. A chimeric HIV-2-HIV-1 peptide fragment comprising said immunodiagnostic peptide fragment of the envelope region of the HIV-2 virus of claim 2 and an immunodiagnostic peptide fragment of the envelope region of the HIV-1 virus.

23. The chimeric HIV-2-HIV-1 peptide fragment of claim 22 wherein said immunodiagnostic peptide fragment of the envelope region of the HIV-1 virus is selected from the group consisting of clone G or peptide fragments derived therefrom.

24. The chimeric HIV-2-HIV-1 peptide fragment of claim 22 wherein said immunodiagnostic peptide fragment of the envelope region of the HIV-1 virus is CBre3.

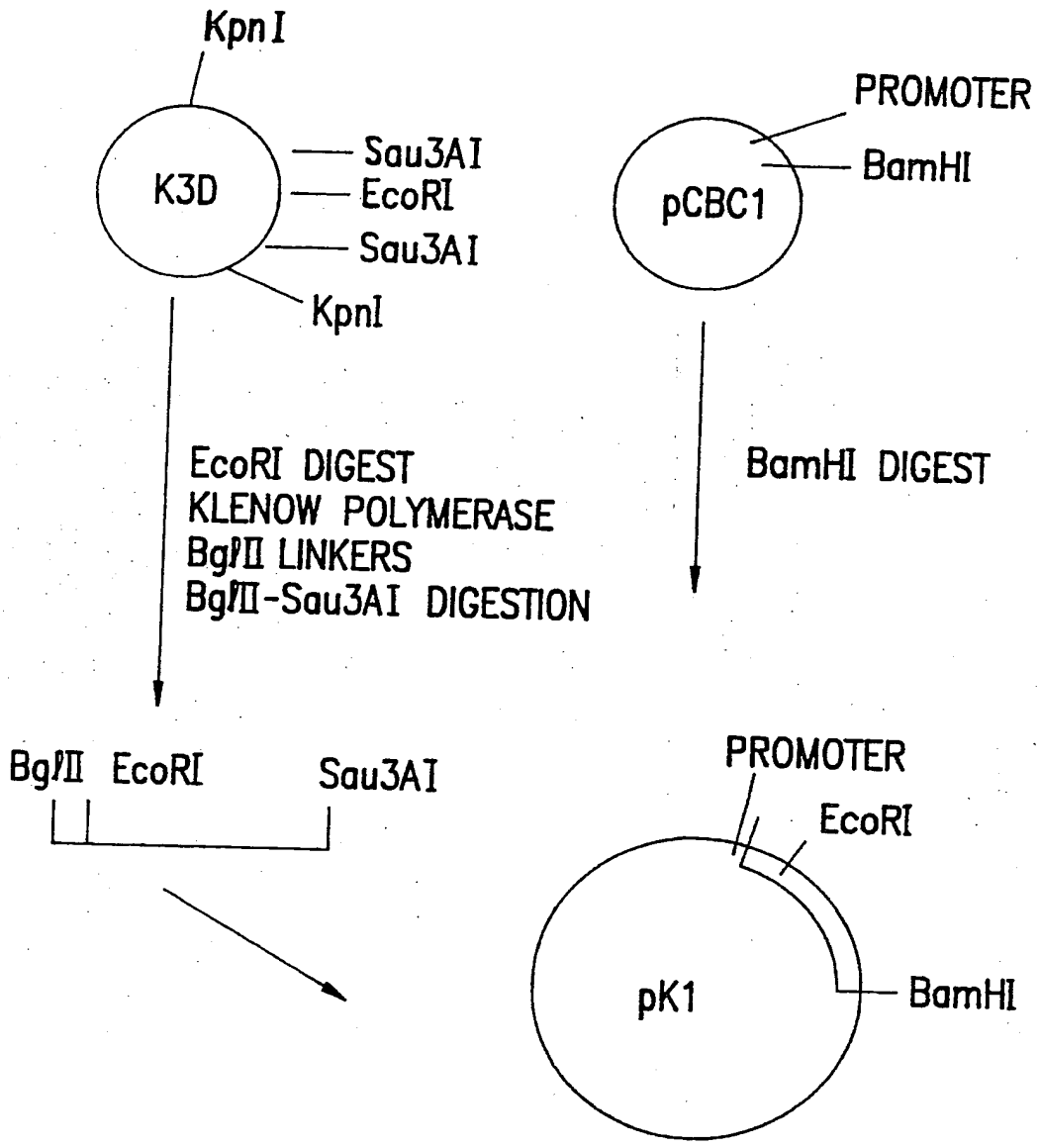

KpnI
K3D
Sau3AI
EcoRI
Sau3AI
KpnI
PROMOTER
BamHI
pCBC1
EcoRI DIGEST
KLENOW POLYMERASE
BglII LINKERS
BglII-Sau3AI DIGESTION
BamHI DIGEST
BglII EcoRI
Sau3AI
PROMOTER
EcoRI
pK1
BamHI

FIG. 1

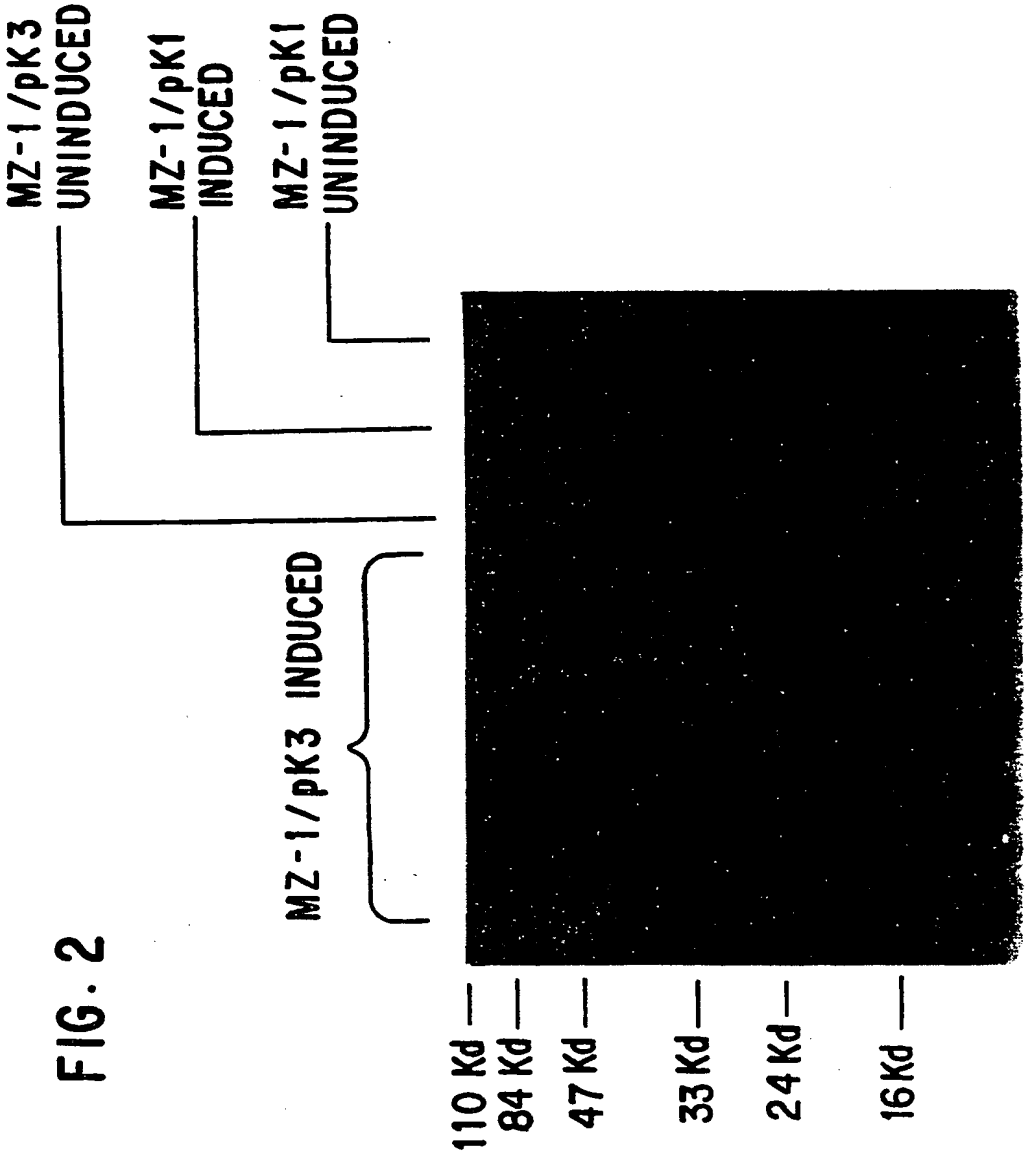
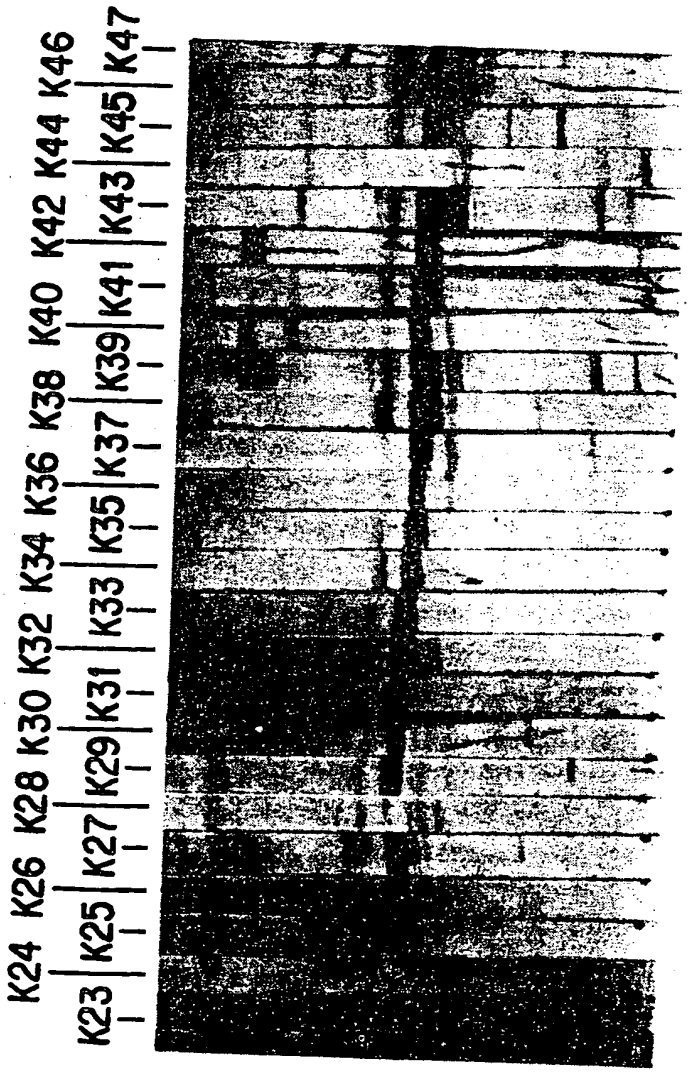


FIG. 2

FIG. 3



```
          10          20          30          40          50          60
          *           *           *           *           *           *
ATG GTT CGT GCA AAC AAA CGC AAC GAG GCT CTA CGA ATC GCG GGA TCT GAA TTC CTC TAT
Met Val Arg Ala Asn Lys Arg Asn Glu Ala Leu Arg Ile Ala Gly Ser Glu Phe Leu Tyr

          70          80          90          100         110         120
          *           *           *           *           *           *
TGC AAC ATG ACT TGG TTC CTT AAT TGG GTA GAA AAC AAG ACG GGT CAA CAG CAT AAC TAT
Cys Asn Met Thr Trp Phe Leu Asn Trp Val Glu Asn Lys Thr Gly Gln Gln His Asn Tyr

          130         140         150         160         170         180
          *           *           *           *           *           *
GTG CCG TGC CAT ATA GAG CAA ATA ATT AAT ACC TGG CAT AAG GTA GGG AAA AAT GTA TAT
Val Pro Cys His Ile Glu Gln Ile Ile Asn Thr Trp His Lys Val Gly Lys Asn Val Tyr

          190         200         210         220         230         240
          *           *           *           *           *           *
TTG CCT CCT AGG GAA GGA GAG TTG TCC TGC GAA TCA ACA GTG ACC AGT ATC ATT GCT AAC
Leu Pro Pro Arg Glu Gly Glu Leu Ser Cys Glu Ser Thr Val Thr Ser Ile Ile Als Asn

          250         260         270         280         290         300
          *           *           *           *           *           *
ATT GAT GTT GAT GGA GAT AAC CGG ACA AAT ATT ACC TTT AGT GCA GAG GTG GCA GAA CTA
Ile Asp Val Asp Gly Asp Asn Arg Thr Asn Ile Thr Phe Ser Ala Glu Val Ala Glu Leu

          310         320         330         340         350         360
          *           *           *           *           *           *
TAC CGA TTG GAA TTG GGG GAT TAT AAA TTA GTA GAA GTA ACA CCA ATT GGC TTC GCC CCT
Tyr Arg Leu Glu Leu Gly Asp Tyr Lys Leu Val Glu Val Thr Pro Ile Gly Phe Ala Pro

          370         380         390         400         410         420
          *           *           *           *           *           *
ACA GCA GAA AAA AGA TAC TCC TCT GCT CCA GGG AGA CAT AAG AGA GGT GTG CTT GTG CTA
Thr Ala Glu Lys Arg Tyr Ser Ser Ala Pro Gly Arg His Lys Arg Gly Val Leu Val Leu

          430         440         450         460         470         480
          *           *           *           *           *           *
GGG TTC CTA GGT TTT CTC ACG ACA GCA GGT GCT GCA ATG GGG GCG GCG TCT CTG ACG CTG
Gly Phe Leu Gly Phe Leu Thr Thr Ala Gly Ala Ala Met Gly Ala Ala Ser Leu Thr Leu
```

FIG. 4

```
          490         500         510         520         530         540
           *           *           *           *           *           *
TCG GCT CAG TCT CGG ACT TTA TTC CGT GGG ATA GTG CAG CAA CAG CAA CAG CTG TTG GAC
Ser Ala Gln Ser Arg Thr Leu Phe Arg Gly Ile Val Gln Gln Gln Gln Leu Leu Asp

          550         560         570         580         590         600
           *           *           *           *           *           *
GTG GTC AAG AGA CAA CAA GAA ATG TTG CGA CTG ACC GTC TGG GGA ACT AAA AAC CTC CAA
Val Val Lys Arg Gln Gln Glu Met Leu Arg Leu Thr Val Trp Gly Thr Lys Asn Leu Gln

          610         620         630         640         650         660
           *           *           *           *           *           *
GCA AGA GTC ACT GCT ATT GAG AAG TAC CTA GCA GAC CAG GCG CGA CTA AAT TCA TGG GGA
Ala Arg Val Thr Ala Ile Glu Lys Tyr Leu Ala Asp Gln Ala Arg Leu Asn Ser Trp Gly

          670         680         690         700         710         720
           *           *           *           *           *           *
TGT GCG TTT AGA CAA GTC TGC CAC ACT ACT GTA CCA TGG GTA AAT GAC ACC TTA ACA CCT
Cys Ala Phe Arg Gln Val Cys His Thr Thr Val Pro Trp Val Asn Asp Thr Leu Thr Pro

          730         740         750         760         770         780
           *           *           *           *           *           *
GAG TGG AAC AAC ATG ACA TGG CAA GAA TGG GAA CAC AAA ATC CGC TTC CTA GAG GCA AAT
Glu Trp Asn Asn Met Thr Trp Gln Glu Trp Glu His Lys Ile Arg Phe Leu Glu Ala Asn

          790         800         810         820         830         840
           *           *           *           *           *           *
ATC AGT GAG AGT TTA GAA CAG GCA CAA ATC CAG CAA GAA AAG AAT ATG TAT GAG CTG CAA
Ile Ser Glu Ser Leu Glu Gln Ala Gln Ile Gln Gln Glu Lys Asn Met Tyr Glu Leu Gln

          850         860         870         880         890         900
           *           *           *           *           *           *
AAG CTA AAT AGC TGG GAT GTT TTT GGC AAT TGG TTT GAC TTA ACC TCC TGG ATC CTA GGT
Lys Leu Asn Ser Trp Asp Val Phe Gly Asn Trp Phe Asp Leu Thr Ser Trp Ile Leu Gly


AAG TAG
Lys ---
```

FIG. 4 (cont.)

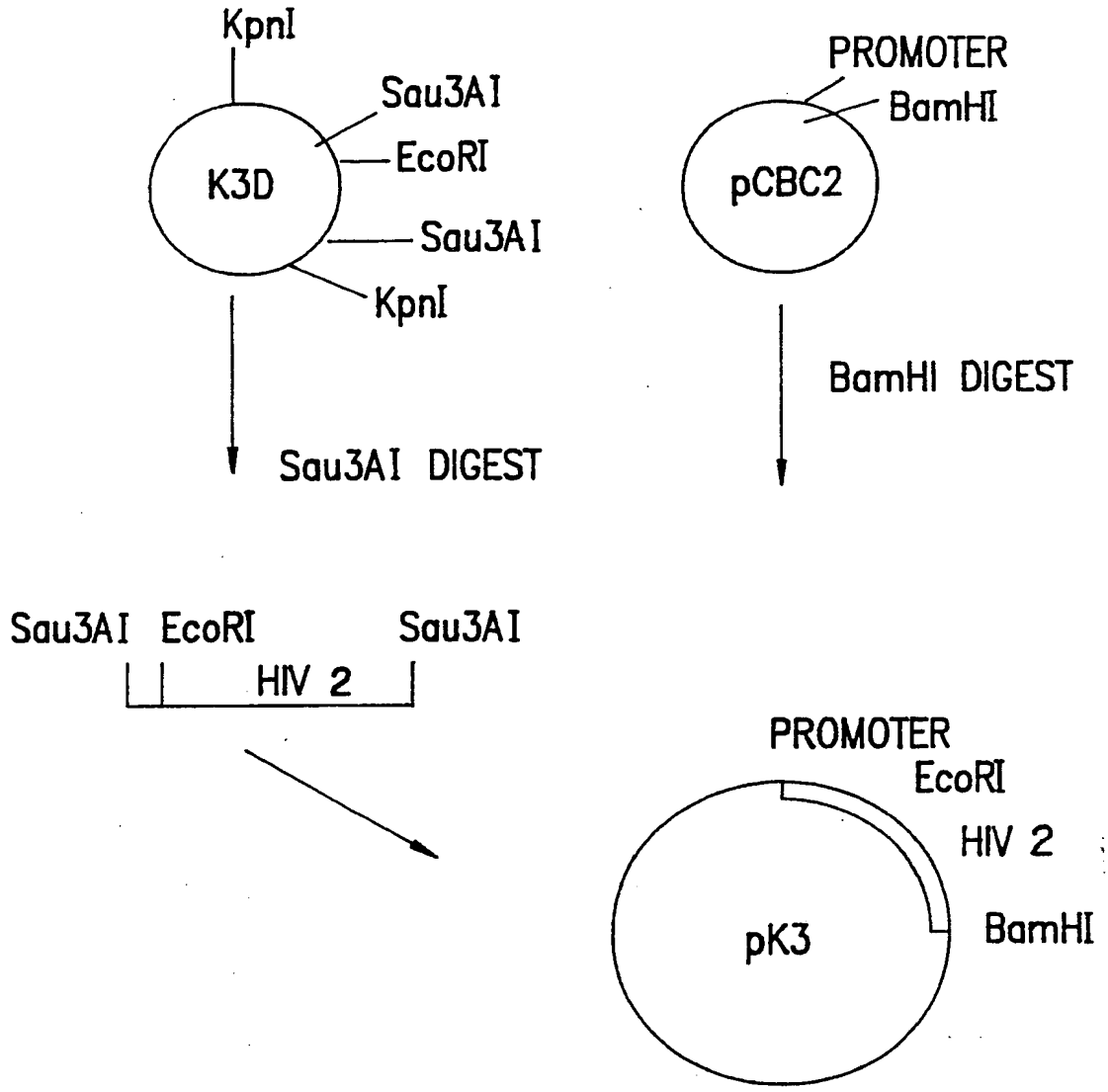


FIG. 5

FIG. 6

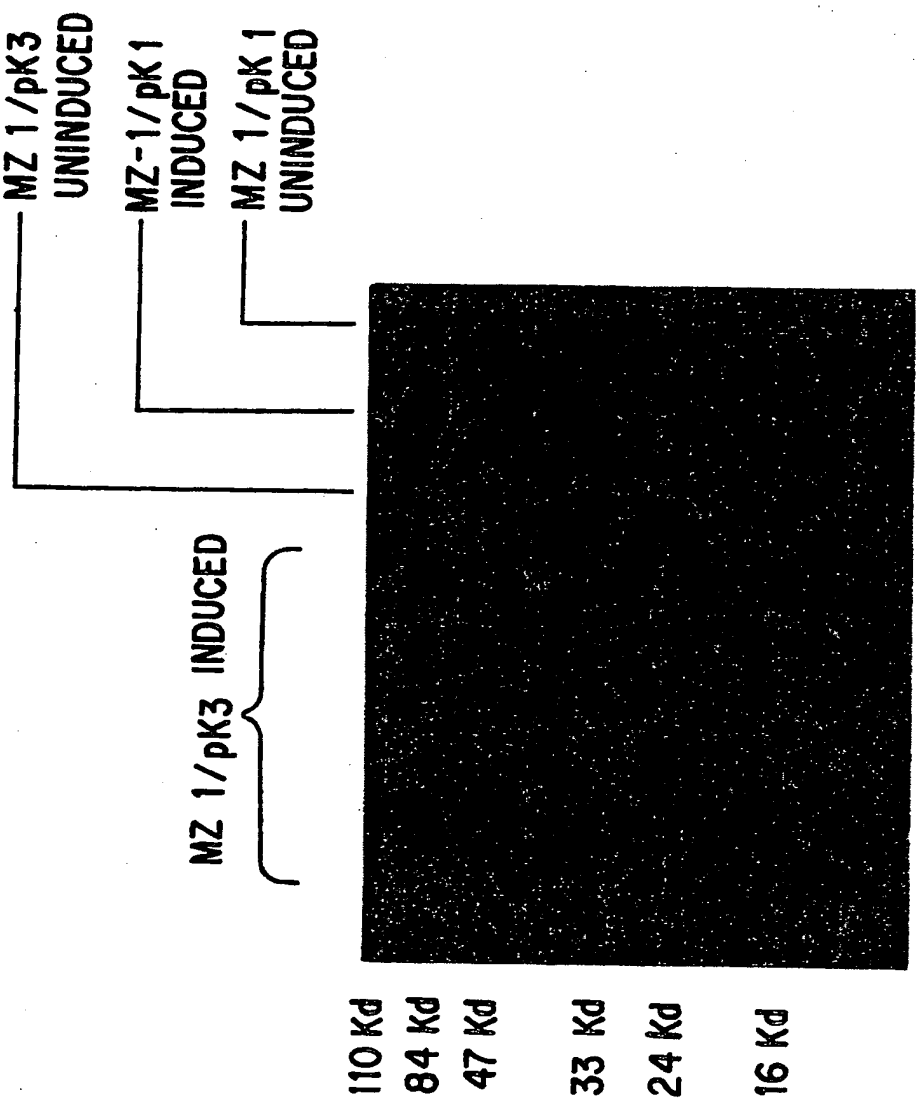
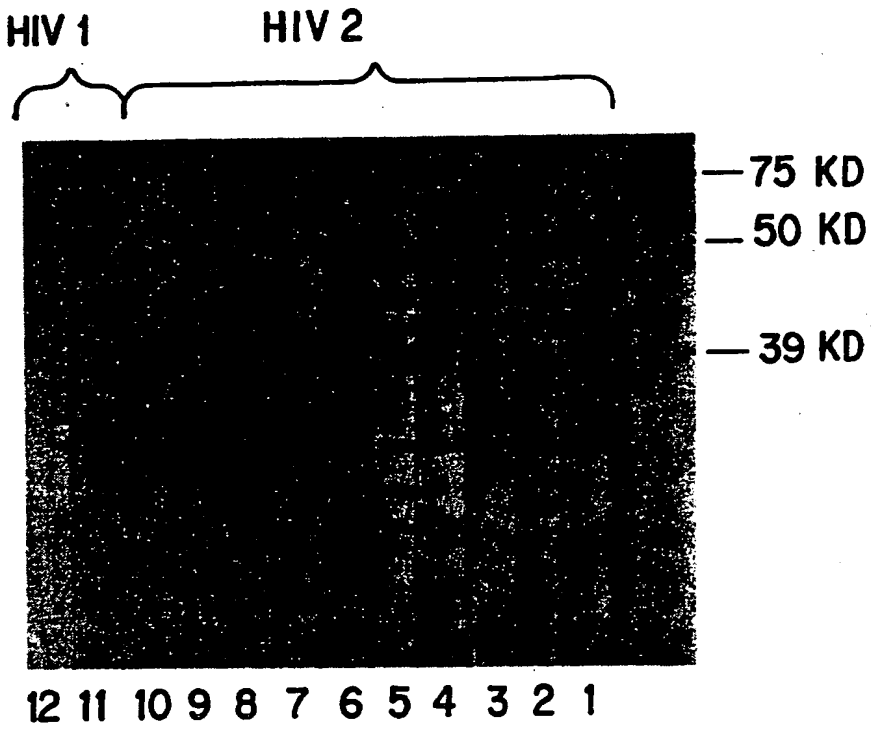


FIG. 7



```
          10         20         30         40         50         60
          *          *          *          *          *          *
ATG GTT CGT GCA AAC AAA CGC AAC GAG GCT CTA CGA ATC GCG CGG ATC ATC AAT AAA AAA
Met Val Arg Ala Asn Lys Arg Asn Glu Ala Leu Arg Ile Ala Arg Ile Ile Asn Lys Lys

          70         80         90         100        110        120
          *          *          *          *          *          *
CCC AGG CAA GCA TGG TGC CGG TTC AAA GGC GAG TGG AGG GAA GCC ATG CAG GAG GTG AAA
Pro Arg Gln Ala Trp Cys Arg Phe Lys Gly Glu Trp Arg Glu Ala Met Gln Glu Val Lys

          130        140        150        160        170        180
          *          *          *          *          *          *
CAA ACC CTT GTA AAA CAT CCC AGG TAT AAA GGA ACC AAT GAC ACA AAT AAA ATT AAC TTT
Gln Thr Leu Val Lys His Pro Arg Tyr Lys Gly Thr Asn Asp Thr Asn Lys Ile Asn Phe

          190        200        210        220        230        240
          *          *          *          *          *          *
ACA GCA CCA GAA AAA GAC TCA GAC CCA GAA GTA GCA TAT ATG TGG ACT AAC TGC AGA GGA
Thr Ala Pro Glu Lys Asp Ser Asp Pro Glu Val Ala Tyr Met Trp Thr Asn Cys Arg Gly

          250        260        270        280        290        300
          *          *          *          *          *          *
GAA TTC CTC TAT TGC AAC ATG ACT TGG TTC CTT AAT TGG GTA GAA AAC AAG ACG GGT CAA
Glu Phe Leu Tyr Cys Asn Met Thr Trp Phe Leu Asn Trp Val Glu Asn Lys Thr Gly Gln

          310        320        330        340        350        360
          *          *          *          *          *          *
CAG CAT AAC TAT GTG CCG TGC CAT ATA GAG CAA ATA ATT AAT ACC TGG CAT AAG GTA GGG
Gln His Asn Tyr Val Pro Cys His Ile Glu Gln Ile Ile Asn Thr Trp His Lys Val Gly

          370        380        390        400        410        420
          *          *          *          *          *          *
AAA AAT GTA TAT TTG CCT CCT AGG GAA GGA GAG TTG TCC TGC GAA TCA ACA GTG ACC AGT
Lys Asn Val Tyr Leu Pro Pro Arg Glu Gly Glu Leu Ser Cys Glu Ser Thr Val Thr Ser
```

FIG. 8

```
          430         440         450         460         470         480
           *           *           *           *           *           *
ATC ATT GCT AAC ATT GAT GTT GAT GGA GAT AAC CGG ACA AAT ATT ACC TTT AGT GCA GAG
Ile Ile Ala Asn Ile Asp Val Asp Gly Asp Asn Arg Thr Asn Ile Thr Phe Ser Ala Glu

          490         500         510         520         530         540
           *           *           *           *           *           *
GTG GCA GAA CTA TAC CGA TTG GAA TTG GGG GAT TAT AAA TTA GTA GAA GTA ACA CCA ATT
Val Ala Glu Leu Tyr Arg Leu Glu Leu Gly Asp Tyr Lys Leu Val Glu Val Thr Pro Ile

          550         560         570         580         590         600
           *           *           *           *           *           *
GGC TTC GCC CCT ACA GCA GAA AAA AGA TAC TCC TCT GCT CCA GGG AGA CAT AAG AGA GGT
Gly Phe Ala Pro Thr Ala Glu Lys Arg Tyr Ser Ser Ala Pro Gly Arg His Lys Arg Gly

          610         620         630         640         650         660
           *           *           *           *           *           *
GTG CTT GTG CTA GGG TTC CTA GGT TTT CTC ACG ACA GCA GGT GCT GCA ATG GGG GCG GCG
Val Leu Val Leu Gly Phe Leu Gly Phe Leu Thr Thr Ala Gly Ala Ala Met Gly Ala Ala

          670         680         690         700         710         720
           *           *           *           *           *           *
TCT CTG ACG CTG TCG GCT CAG TCT CGG ACT TTA TTC CGT GGG ATA GTG CAG CAA CAG CAA
Ser Leu Thr Leu Ser Ala Gln Ser Arg Thr Leu Phe Arg Gly Ile Val Gln Gln Gln Gln

          730         740         750         760         770         780
           *           *           *           *           *           *
CAG CTG TTG GAC GTG GTC AAG AGA CAA CAA GAA ATG TTG CGA CTG ACC GTC TGG GGA ACT
Gln Leu Leu Asp Val Val Lys Arg Gln Gln Glu Met Leu Arg Leu Thr Val Trp Gly Thr
```

FIG. 8 (cont.)

```
          790         800         810         820         830         840
           *           *           *           *           *           *
AAA AAC CTC CAG GCA AGA GTC ACT GCT ATT GAG AAG TAC CTA GCA GAC CAG GCG CGA CTA
Lys Asn Leu Gln Ala Arg Val Thr Ala Ile Glu Lys Tyr Leu Ala Asp Gln Ala Arg Leu

          850         860         870         880         890         900
           *           *           *           *           *           *
AAT TCA TGG GGA TGT GCG TTT AGA CAA GTC TGC CAC ACT ACT GTA CCA TGG GTA AAT GAC
Asn Ser Trp Gly Cys Ala Phe Arg Gln Val Cys His Thr Thr Val Pro Trp Val Asn Asp

          910         920         930         940         950         960
           *           *           *           *           *           *
ACC TTA ACA CCT GAG TGG AAC AAC ATG ACA TGG CAA GAA TGG GAA CAC AAA ATC CGC TTC
Thr Leu Thr Pro Glu Trp Asn Asn Met Thr Trp Gln Glu Trp Glu His Lys Ile Arg Phe

          970         980         990        1000        1010        1020
           *           *           *           *           *           *
CTA GAG GCA AAT ATC AGT GAG AGT TTA GAA CAG GCA CAA ATC CAG CAA GAA AAG AAT ATG
Leu Glu Ala Asn Ile Ser Glu Ser Leu Glu Gln Ala Gln Ile Gln Gln Glu Lys Asn Met

         1030        1040        1050        1060        1070        1080
           *           *           *           *           *           *
TAT GAG CTG CAA AAG CTA AAT AGC TGG GAT GTT TTT GGC AAT TGG TTT GAC TTA ACC TCC
Tyr Glu Leu Gln Lys Leu Asn Ser Trp Asp Val Phe Gly Asn Trp Phe Asp Leu Thr Ser

         1090
           *
TGG ATC CTA GGT AAG TAG
Trp Ile Leu Gly Lys ---
```

FIG. 8 (cont.)

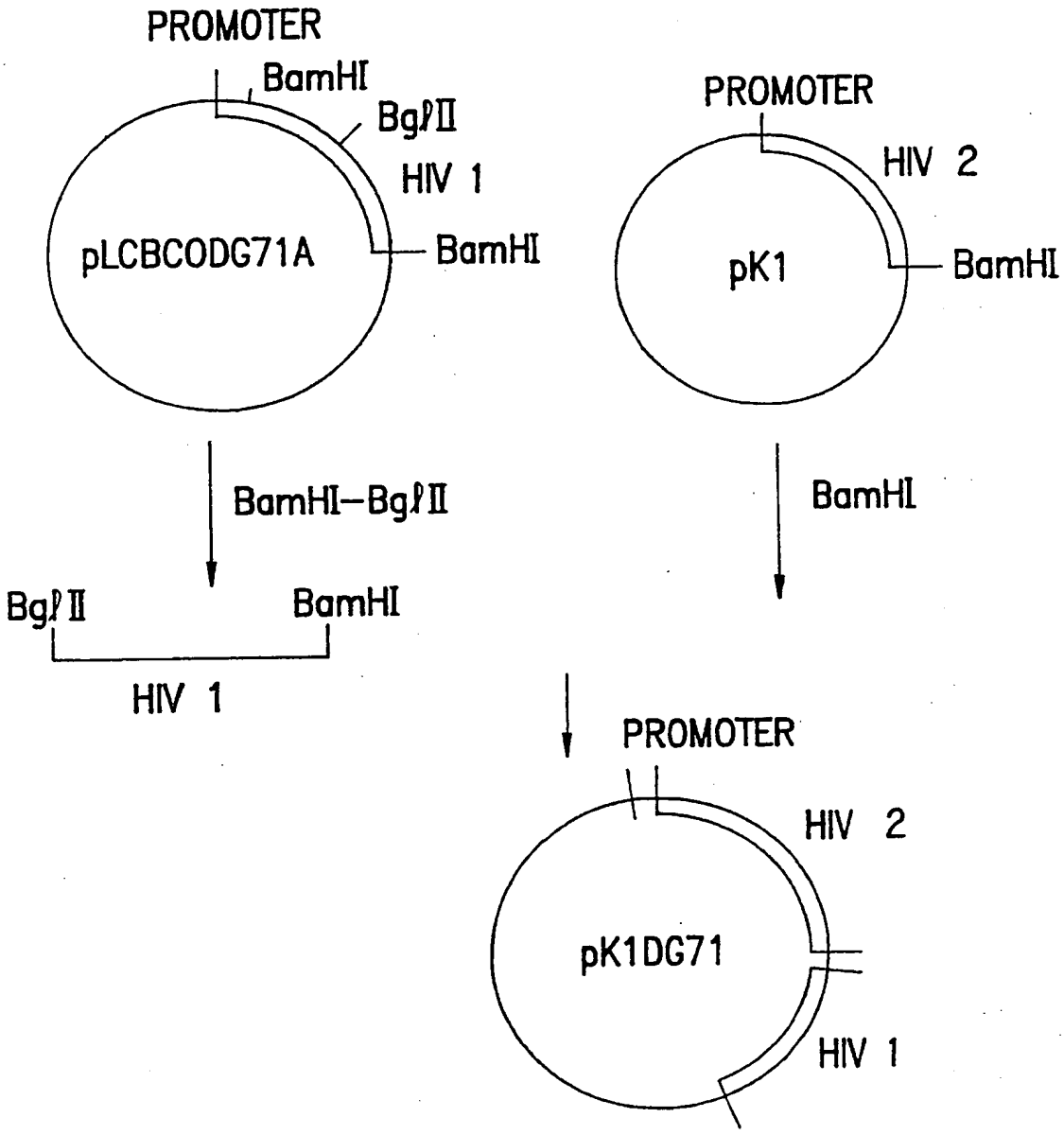


FIG. 9

FIG. 10

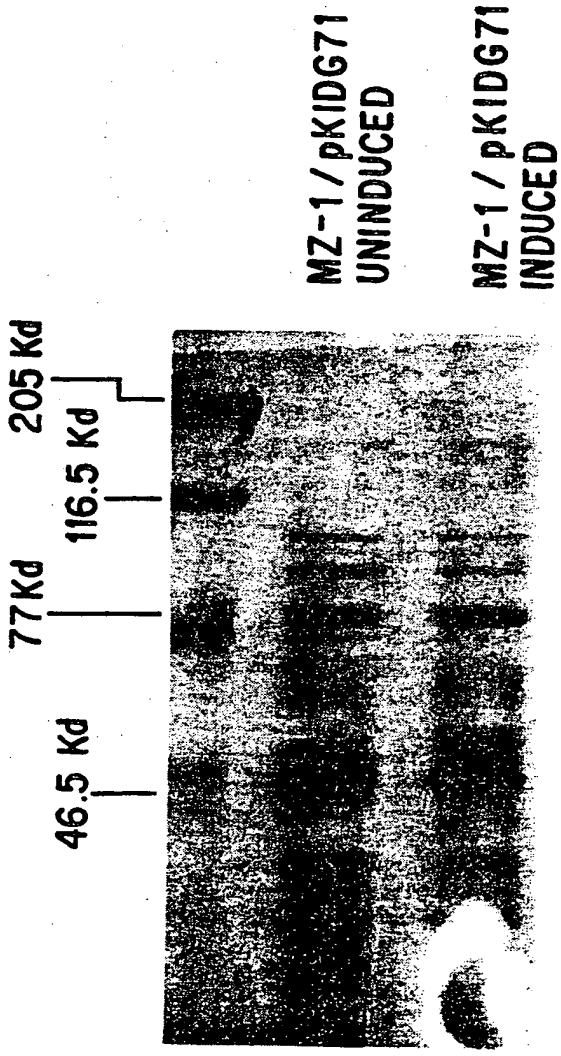

MZ-1 / pKIDG71
UNINDUCED
MZ-1 / pKIDG71
INDUCED
205 Kd
116.5 Kd
77Kd
46.5 Kd

FIG. 11A

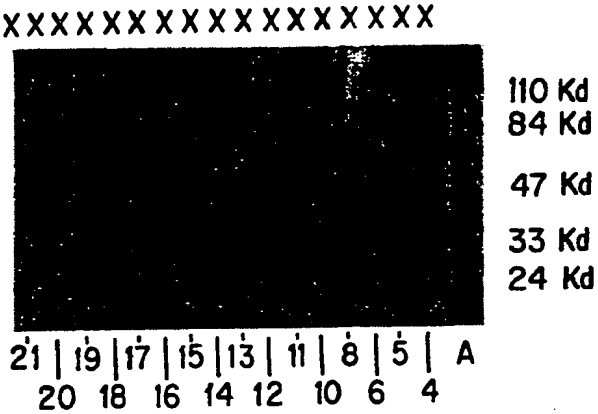


FIG. 11B

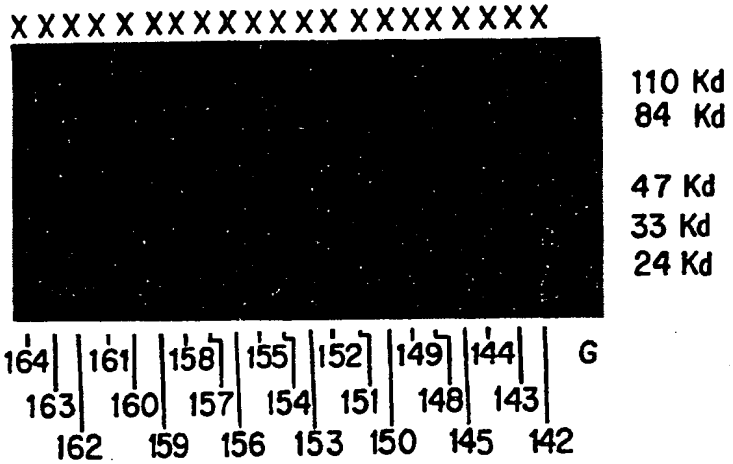
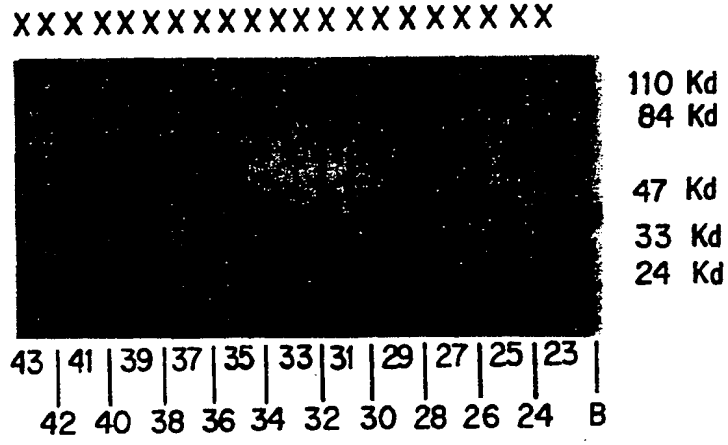


FIG. 11C



SUBSTITUTE SHEET

```
          10         20         30         40         50         60
           *          *          *          *          *          *
ATG GTT CGT GCA AAC AAA CGC AAC GAG GCT CTA CGA ATC GCG GGA TCT GAA TTC CTC TAT
Met Val Arg Ala Asn Lys Arg Asn Glu Ala Leu Arg Ile Ala Gly Ser Glu Phe Leu Tyr

          70         80         90        100        110        120
           *          *          *          *          *          *
TGC AAC ATG ACT TGG TTC CTT AAT TGG GTA GAA AAC AAG ACG GGT CAA CAG CAT AAC TAT
Cys Asn Met Thr Trp Phe Leu Asn Trp Val Glu Asn Lys Thr Gly Gln Gln His Asn Tyr

         130        140        150        160        170        180
           *          *          *          *          *          *
GTG CCG TGC CAT ATA GAG CAA ATA ATT AAT ACC TGG CAT AAG GTA GGG AAA AAT GTA TAT
Val Pro Cys His Ile Glu Gln Ile Ile Asn Thr Trp His Lys Val Gly Lys Asn Val Tyr

         190        200        210        220        230        240
           *          *          *          *          *          *
TTG CCT CCT AGG GAA GGA GAG TTG TCC TGC GAA TCA ACA GTG ACC AGT ATC ATT GCT AAC
Leu Pro Pro Arg Glu Gly Glu Leu Ser Cys Glu Ser Thr Val Thr Ser Ile Ile Ala Asn

         250        260        270        280        290        300
           *          *          *          *          *          *
ATT GAT GTT GAT GGA GAT AAC CGG ACA AAT ATT ACC TTT AGT GCA GAG GTG GCA GAA CTA
Ile Asp Val Asp Gly Asp Asn Arg Thr Asn Ile Thr Phe Ser Ala Glu Val Ala Glu Leu

         310        320        330        340        350        360
           *          *          *          *          *          *
TAC CGA TTG GAA TTG GGG GAT TAT AAA TTA GTA GAA GTA ACA CCA ATT GGC TTC GCC CCT
Tyr Arg Leu Glu Leu Gly Asp Tyr Lys Leu Val Glu Val Thr Pro Ile Gly Phe Ala Pro

         370        380        390        400        410        420
           *          *          *          *          *          *
ACA GCA GAA AAA AGA TAC TCC TCT GCT CCA GGG AGA CAT AAG AGA GGT GTG CTT GTG CTA
Thr Ala Glu Lys Arg Tyr Ser Ser Ala Pro Gly Arg His Lys Arg Gly Val Leu Val Leu

         430        440        450        460        470        480
           *          *          *          *          *          *
GGG TTC CTA GGT TTT CTC ACG ACA GCA GGT GCT GCA ATG GGG GCG GCG TCT CTG ACG CTG
Gly Phe Leu Gly Phe Leu Thr Thr Ala Gly Ala Ala Met Gly Ala Ala Ser Leu Thr Leu
```

FIG. 12

SUBSTITUTE SHEET

```
          490         500         510         520         530         540
           *           *           *           *           *           *
TCG GCT CAG TCT CGG ACT TTA TTC CGT GGG ATA GTG CAG CAA CAG CAA CAG CTG TTG GAC
Ser Ala Gln Ser Arg Thr Leu Phe Arg Gly Ile Val Gln Gln Gln Gln Leu Leu Asp

          550         560         570         580         590         600
           *           *           *           *           *           *
GTG GTC AAG AGA CAA CAA GAA ATG TTG CGA CTG ACC GTC TGG GGA ACT AAA AAC CTC CAG
Val Val Lys Arg Gln Gln Glu Met Leu Arg Leu Thr Val Trp Gly Thr Lys Asn Leu Gln

          610         620         630         640         650         660
           *           *           *           *           *           *
GCA AGA GTC ACT GCT ATT GAG AAG TAC CTA GCA GAC CAG GCG CGA CTA AAT TCA TGG GGA
Ala Arg Val Thr Ala Ile Glu Lys Tyr Leu Ala Asp Gln Ala Arg Leu Asn Ser Trp Gly

          670         680         690         700         710         720
           *           *           *           *           *           *
TGT GCG TTT AGA CAA GTC TGC CAC ACT ACT GTA CCA TGG GTA AAT GAC ACC TTA ACA CCT
Cys Ala Phe Arg Gln Val Cys His Thr Thr Val Pro Trp Val Asn Asp Thr Leu Thr Pro

          730         740         750         760         770         780
           *           *           *           *           *           *
GAG TGG AAC AAC ATG ACA TGG CAA GAA TGG GAA CAC AAA ATC CGC TTC CTA GAG GCA AAT
Glu Trp Asn Asn Met Thr Trp Gln Glu Trp Glu His Lys Ile Arg Phe Leu Glu Ala Asn

          790         800         810         820         830         840
           *           *           *           *           *           *
ATC AGT GAG AGT TTA GAA CAG GCA CAA ATC CAG CAA GAA AAG AAT ATG TAT GAG CTG CAA
Lys Leu Asn Ser Trp Asp Val Phe Gly Asn Trp Phe Asp Leu Thr Ser Trp Ile Phe Arg

          850         860         870         880         890         900
           *           *           *           *           *           *
AAG CTA AAT AGC TGG GAT GTT TTT GGC AAT TGG TTT GAC TTA ACC TCC TGG ATC TTC AGA
Lys Leu Asn Ser Trp Asp Val Phe Gly Asn Trp Phe Asp Leu Thr Ser Trp Ile Phe Arg

          910         920         930         940         950         960
           *           *           *           *           *           *
CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA TAT AAA TAT AAA GTA GTA
Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val
```

FIG. 12 (cont.)

SUBSTITUTE SHEET

```
          970         980         990        1000        1010        1020
           *           *           *           *           *           *
AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC AAG GCA AAG AGA AGA GTG GTG CAG AGA GAA
Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu

         1030        1040        1050        1060        1070        1080
           *           *           *           *           *           *
AAA AGA GCA GTG GGA ATA GGA CAG GCC AGA CAA TTA TTG TCT GGT ATA GTG CAG CAG CAG
Lys Arg Ala Val Gly Ile Gly Gln Ala Arg Gln Leu Leu Ser Gly Ile Val Gln Gln Gln

         1090        1100        1110        1120        1130        1140
           *           *           *           *           *           *
AAC AAT TTG CTG AGG GCT ATT GAG GGC CAA CAG CAT CTG TTG CAA CTC ACA GTC TGG GGC
Asn Asn Leu Leu Arg Ala Ile Glu Gly Gln Gln His Leu Leu Gln Leu Thr Val Trp Gly

         1150        1160        1170        1180        1190        1200
           *           *           *           *           *           *
ATC AAG CAG CTC CAG GCA AGA ATC CTG GCT GTG GAA AGA TAC CTA AAG GAT CAA CAG CTC
Ile Lys Gln Leu Gln Ala Arg Ile Leu Ala Val Glu Arg Tyr Leu Lys Asp Gln Gln Leu

         1210        1220        1230        1240        1250        1260
           *           *           *           *           *           *
CTG GGG ATT TGG GGT TGC TCT GGA AAA CTC ATT TGC ACC ACT GCT GTG CCT TGG AAT GCT
Leu Gly Ile Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala

         1270        1280        1290        1300        1310        1320
           *           *           *           *           *           *
AGT TGG AGT AAT AAA TCT CTG GAA CAG ATT TGG AAT AAC ATG ACC TGG ATG GAG TGG GAC
Ser Trp Ser Asn Lys Ser Leu Glu Gln Ile Trp Asn Asn Met Thr Trp Met Glu Trp Asp

         1330        1340        1350        1360        1370        1380
           *           *           *           *           *           *
AGA GAA ATT AAC AAT TAC ACA AGC TTA ATA CAC TCC TTA ATT GAA GAA TCG CAA AAC CAG
Arg Glu Ile Asn Asn Tyr Thr Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln

         1390        1400        1410        1420        1430        1440
           *           *           *           *           *           *
CAA GAA AAG AAT GAA CAA GAA TTA TTG GAA TTA GAT AAA TGG GCA CGG ATC CTA GGT AAG
Gln Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Arg Ile Leu Gly Lys
```

FIG. 12 (cont.)

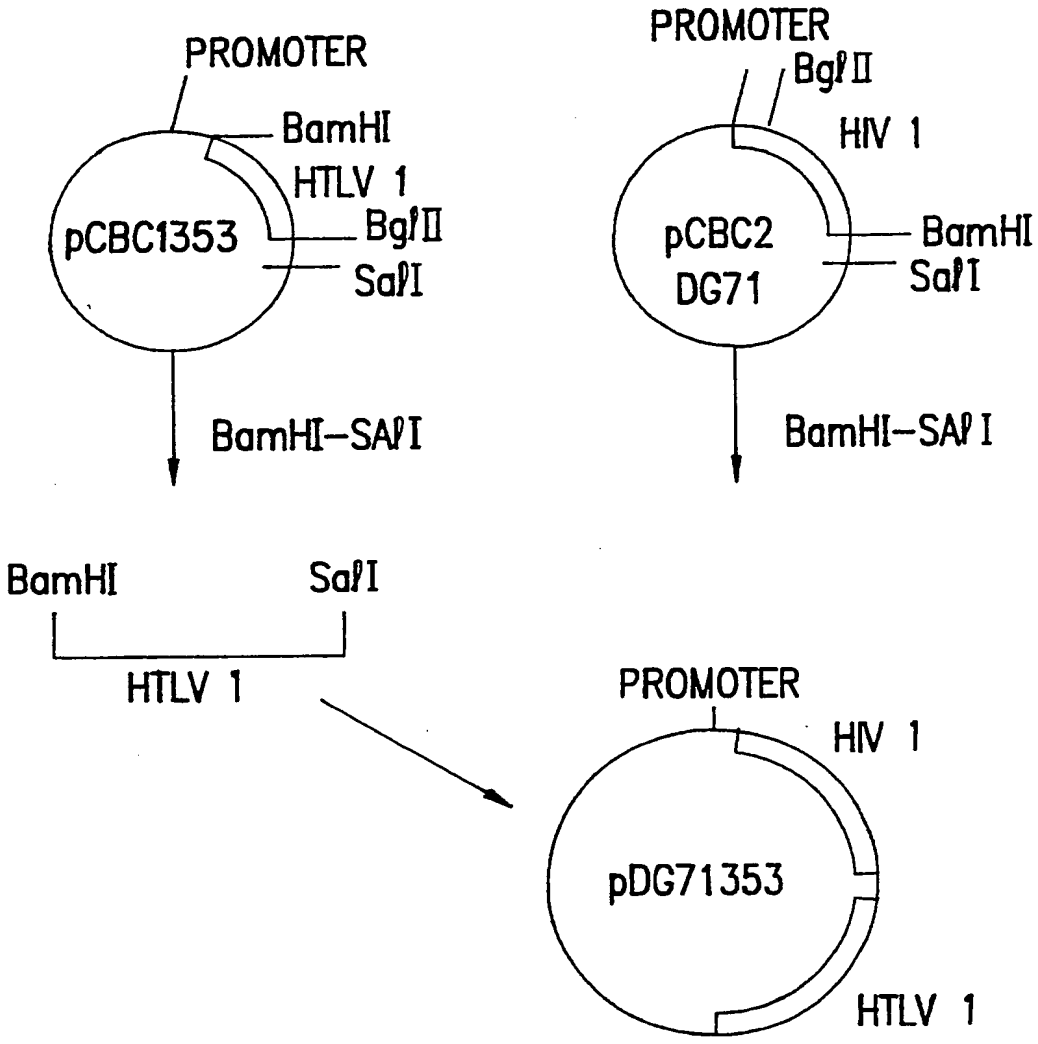


FIG. 13

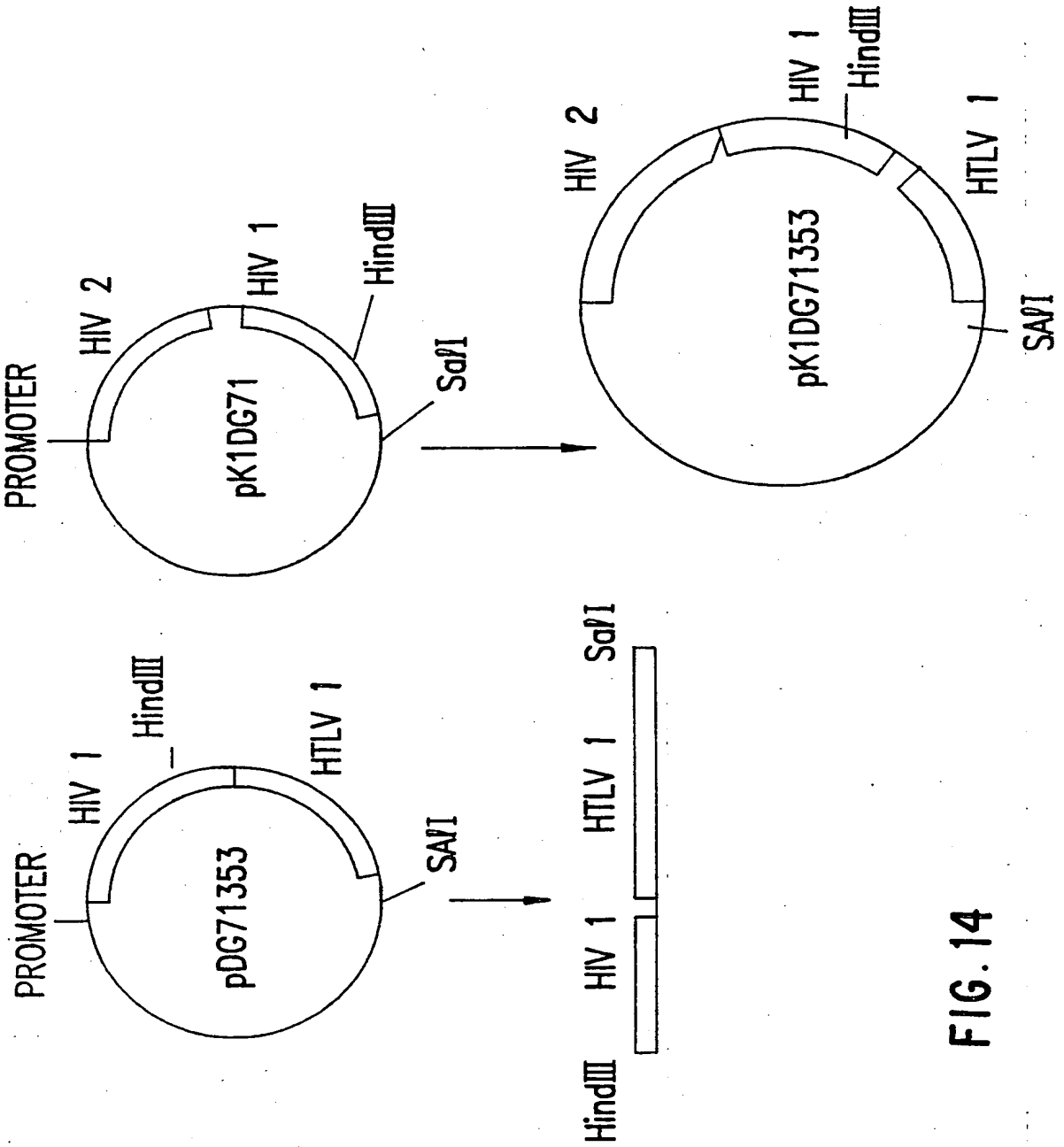


FIG. 14

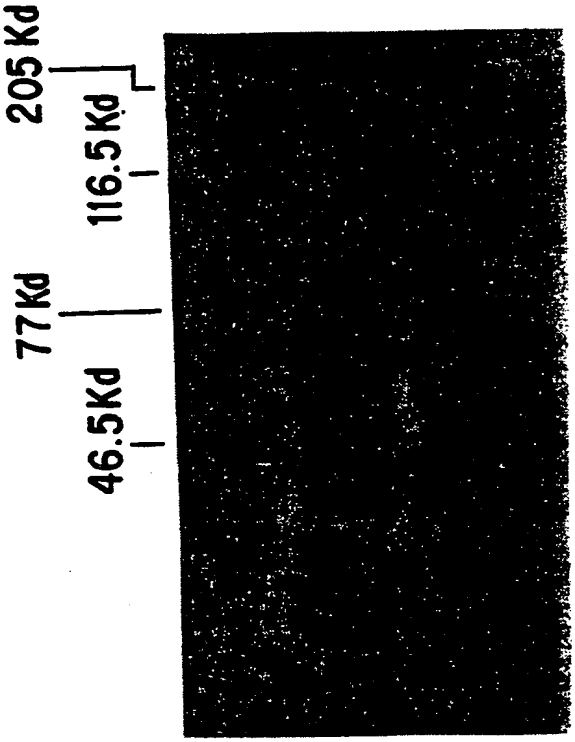
```
          10          20          30          40          50          60
          *           *           *           *           *           *
ATG GTT CGT GCA AAC AAA CGC AAC GAG GCT CTA CGA ATC GCG CGG ATC TTC AGA CCT GGA
Met Val Arg Ala Asn Lys Arg Asn Glu Ala Leu Arg Ile Ala Arg Ile Phe Arg Pro Gly

          70          80          90          100         110         120
          *           *           *           *           *           *
GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA TAT AAA TAT AAA GTA GTA AAA ATT
Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile

          130         140         150         160         170         180
          *           *           *           *           *           *
GAA CCA TTA GGA GTA GCA CCC ACC AAG GCA AAG AGA AGA GTG GTG CAG AGA GAA AAA AGA
Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg

          190         200         210         220         230         240
          *           *           *           *           *           *
GCA GTG GGA ATA GGA CAG GCC AGA CAA TTA TTG TCT GGT ATA GTG CAG CAG CAG AAC AAT
Ala Val Gly Ile Gly Gln Ala Arg Gln Leu Leu Ser Gly Ile Val Gln Gln Gln Asn Asn

          250         260         270         280         290         300
          *           *           *           *           *           *
TTG CTG AGG GCT ATT GAG GGC CAA CAG CAT CTG TTG CAA CTC ACA GTC TGG GGC ATC AAG
Leu Leu Arg Ala Ile Glu Gly Gln Gln His Leu Leu Gln Leu Thr Val trp Gly Ile Lys

          310         320         330         340         350         360
          *           *           *           *           *           *
CAG CTC CAG GCA AGA ATC CTG GCT GTG GAA AGA TAC CTA AAG GAT CAA CAG CTC CTG GGG
Gln Leu Gln Ala Arg Ile Leu Ala Val Glu Arg Tyr Leu Lys Asp Gln Gln Leu Leu Gly

          370         380         390         400         410         420
          *           *           *           *           *           *
ATT TGG GGT TGC TCT GGA AAA CTC ATT TGC ACC ACT GCT GTG CCT TGG AAT GCT AGT TGG
Ile Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala Ser Trp

          430         440         450         460         470         480
          *           *           *           *           *           *
AGT AAT AAA TCT CTG GAA CAG ATT TGG AAT AAC ATG ACC TGG ATG GAG TGG GAC AGA GAA
Ser Asn Lys Ser Leu Glu Gln Ile Trp Asn Asn Met Thr Trp Met Glu Trp Asp Arg Glu
```

FIG. 15

```
          490         500         510         520         530         540
           *           *           *           *           *           *
ATT AAC AAT TAC ACA AGC TTA ATA CAC TCC TTA ATT GAA GAA TCG CAA AAC CAG CAA GAA
Ile Asn Asn Tyr Thr Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu

          550         560         570         580         590         600
           *           *           *           *           *           *
AAG AAT GAA CAA GAA TTA TTG GAA TTA GAT AAA TGG GCA CGG ATC GAA GAT CTT CGA TCC
Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Arg Ile Glu Asp Leu Arg Ser

          610         620         630         640         650         660
           *           *           *           *           *           *
CGC TCC CGC CGA GCG GCT GGC GGG ATT ACC GGC TCC ATG TCC CTC GCC TCA GGA AAG AGC
Arg Ser Arg Arg Ala Ala Gly Gly Ile Thr Gly Ser Met Ser Leu Ala Ser Gly Lys Ser

          670         680         690         700         710         720
           *           *           *           *           *           *
CTC CTA CAT GAG GTG GAC AAA GAT ATT TCC CAG TTA ACT CAA GCA ATA GTC AAA AAC CAC
Leu Leu His Glu Val Asp Lys Asp Ile Ser Gln Leu Thr Gln Ala Ile Val Lys Asn His

          730         740         750         760         770         780
           *           *           *           *           *           *
AAA AAT CTA CTC AAA ATT GCG CAG TAT GCT GCC CAG AAC AGA CGA GGC CTT GAT CTC CTG
Lys Asn Leu Leu Lys Ile Ala Gln Tyr Ala Ala Gln Asn Arg Arg Gly Leu Asp Leu Leu

          790         800         810         820         830         840
           *           *           *           *           *           *
TTC TGG GAG CAA GGA GGA TTA TGC AAA GCA TTA CAA GAA CAG TGC CGT TTT CCG AAT ATT
Phe Trp Glu Gln Gly Gly Leu Cys Lys Ala Leu Gln Glu Gln Cys Arg Phe Pro Asn Ile

          850         860         870         880         890         900
           *           *           *           *           *           *
ACC AAT TCC CAT GTC CCA ATA CTA CAA GAA AGA CCC CCC CTT GAG AAT CGA GTC CTG ACT
Thr Asn Ser His Val Pro Ile Leu Gln Glu Arg Pro Pro Leu Glu Asn Arg Val Leu Thr

          910         920         930         940         950
           *           *           *           *           *
GGC TGG GGC CTT AAC TGG GAC CTT GGC CTC TCA CAG TGG GCT CGA TCC TAG
Gly Trp Gly Leu Asn Trp Asn Leu Gly Leu Ser Gln Trp Ala Arg Ser ---
```

FIG. 15 (cont.)

FIG. 16



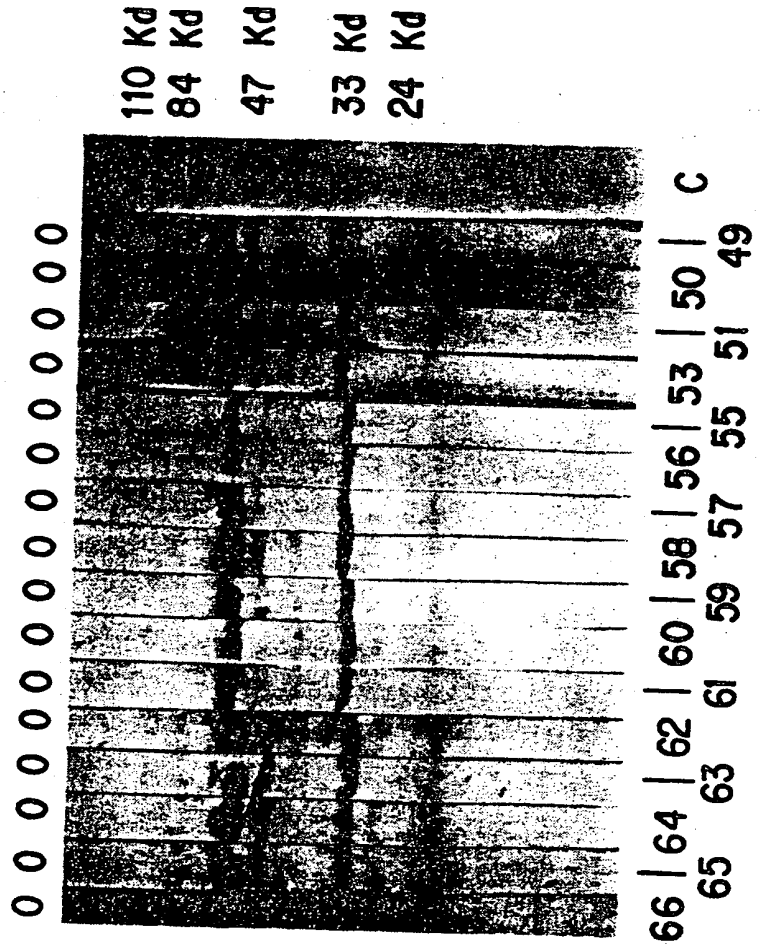
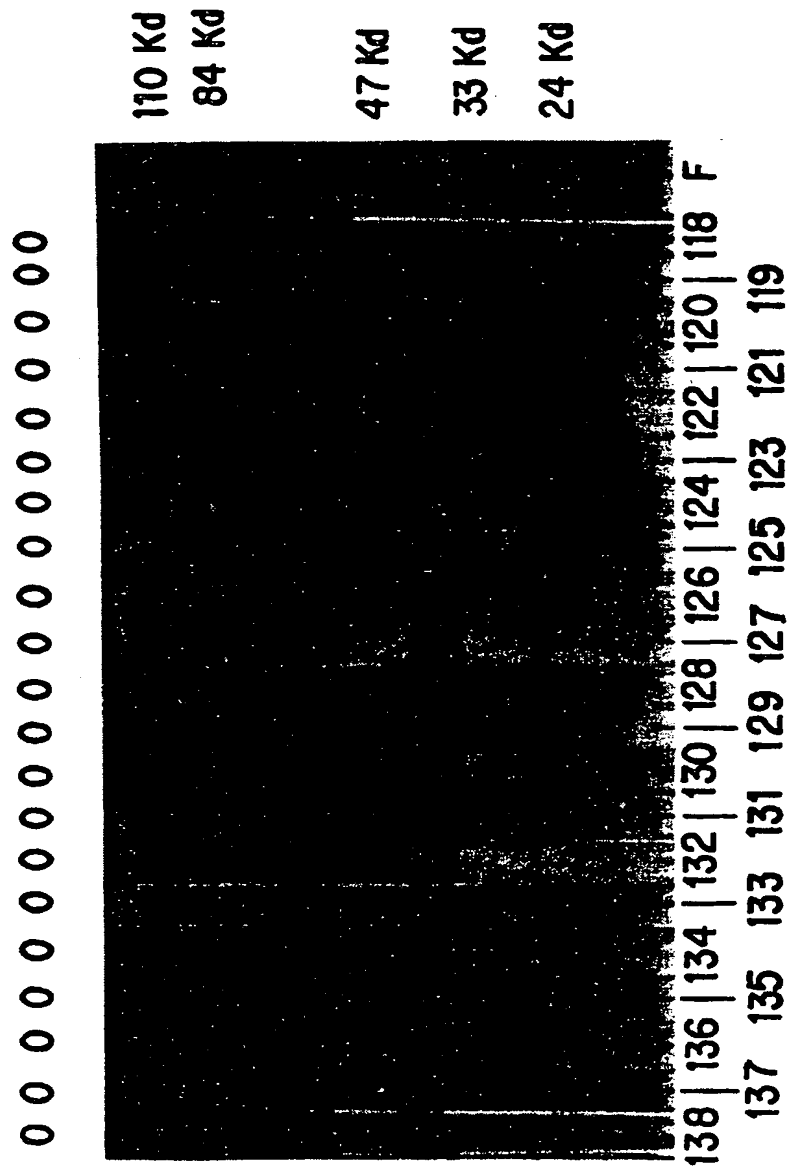


FIG. 17

FIG. 18



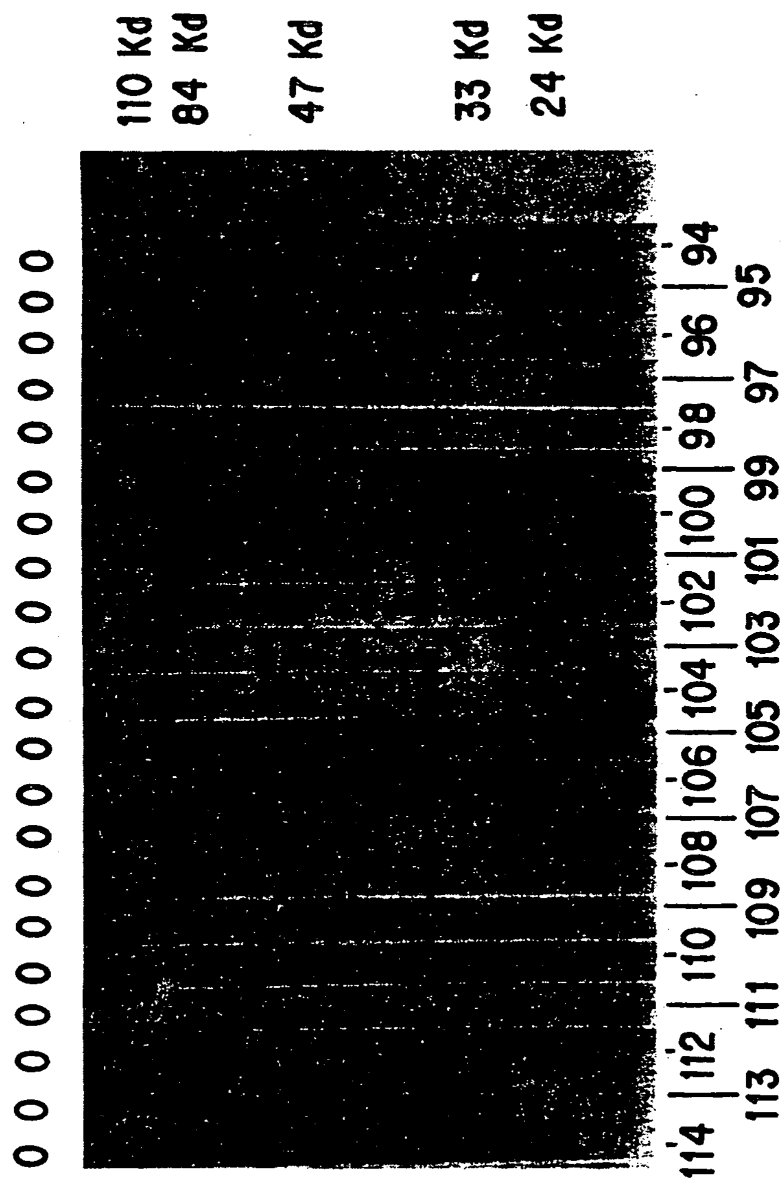


FIG. 19

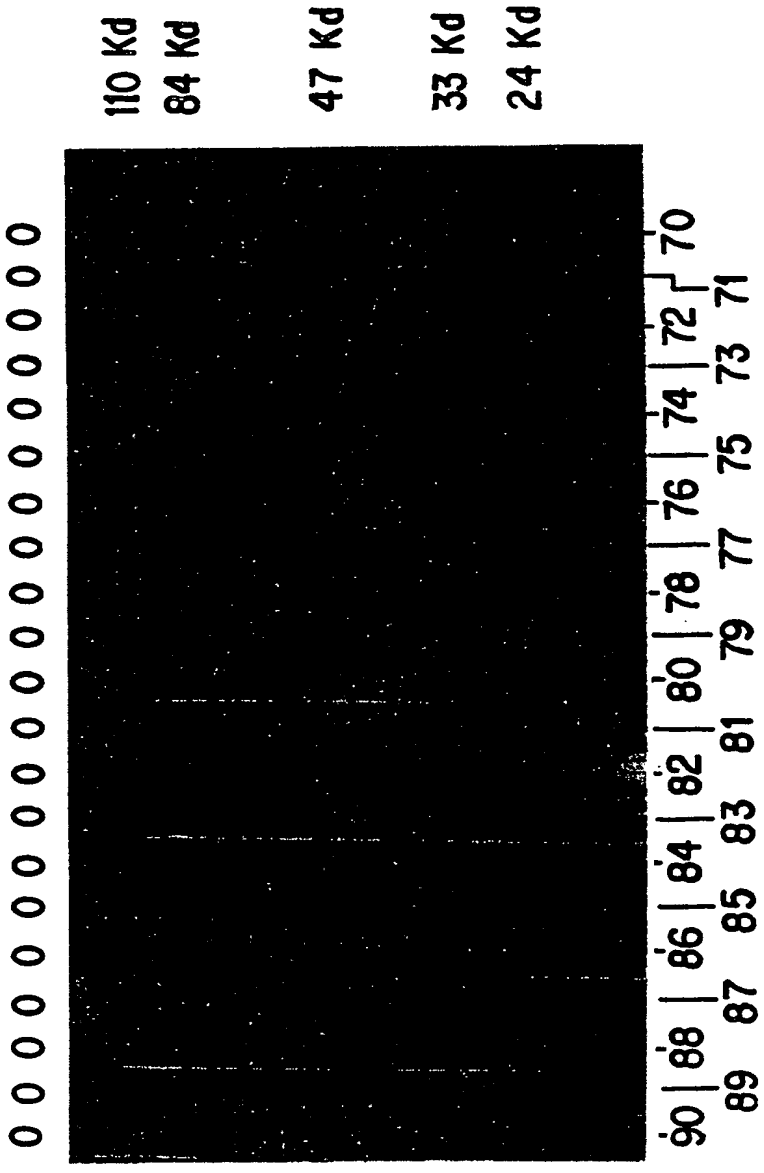


FIG. 20

```
          10         20         30         40         50         60
          *          *          *          *          *          *
ATG GTT CGT GCA AAC AAA CGC AAC GAG GCT CTA CGA ATC GCG GGA TCT GAA TTC CTC TAT
Met Val Arg Ala Asn Lys Arg Asn Glu Ala Leu Arg Ile Ala Gly Ser Glu Phe Leu Tyr

          70         80         90         100        110        120
          *          *          *          *          *          *
TGC AAC ATG ACT TGG TTC CTT AAT TGG GTA GAA AAC AAG ACG GGT CAA CAG CAT AAC TAT
Cys Asn Met Thr Trp Phe Leu Asn Trp Val Glu Asn Lys Thr Gly Gln Gln His Asn Tyr

          130        140        150        160        170        180
          *          *          *          *          *          *
GTG CCG TGC CAT ATA GAG CAA ATA ATT AAT ACC TGG CAT AAG GTA GGG AAA AAT GTA TAT
Val Pro Cys His Ile Glu Gln Ile Ile Asn Thr Trp His Lys Val Gly Lys Asn Val Tyr

          190        200        210        220        230        240
          *          *          *          *          *          *
TTG CCT CCT AGG GAA GGA GAG TTG TCC TGC GAA TCA ACA GTG ACC AGT ATC ATT GCT AAC
Leu Pro Pro Arg Glu Gly Glu Leu Ser Cys Glu Ser Thr Val Thr Ser Ile Ile Ala Asn

          250        260        270        280        290        300
          *          *          *          *          *          *
ATT GAT GTT GAT GGA GAT AAC CGG ACA AAT ATT ACC TTT AGT GCA GAG GTG GCA GAA CTA
Ile Asp Val Asp Gly Asp Asn Arg Thr Asn Ile Thr Phe Ser Ala Glu Val Ala Glu Leu

          310        320        330        340        350        360
          *          *          *          *          *          *
TAC CGA TTG GAA TTG GGG GAT TAT AAA TTA GTA GAA GTA ACA CCA ATT GGC TTC GCC CCT
Tyr Arg Leu Glu Leu Gly Asp Tyr Lys Leu Val Glu Val Thr Pro Ile Gly Phe Ala Pro

          370        380        390        400        410        420
          *          *          *          *          *          *
ACA GCA GAA AAA AGA TAC TCC TCT GCT CCA GGG AGA CAT AAG AGA GGT GTG CTT GTG CTA
Thr Ala Glu Lys Arg Tyr Ser Ser Ala Pro Gly Arg His Lys Arg Gly Val Leu Val Leu
```

FIG. 21

```
          430         440         450         460         470         480
           *           *           *           *           *           *
GGG TTC CTA GGT TTT CTC ACG ACA GCA GGT GCT GCA ATG GGG GCG GCG TCT CTG ACG CTG
Gly Phe Leu Gly Phe Leu Thr Thr Ala Gly Ala Ala Met Gly Ala Ala Ser Leu Thr Leu

          490         500         510         520         530         540
           *           *           *           *           *           *
TCG GCT CAG TCT CGG ACT TTA TTC CGT GGG ATA GTG CAG CAA CAG CAA CAG CTG TTG GAC
Ser Ala Gln Ser Arg Thr Leu Phe Arg Gly Ile Val Gln Gln Gln Gln Leu Leu Asp

          550         560         570         580         590         600
           *           *           *           *           *           *
GTG GTC AAG AGA CAA CAA GAA ATG TTG CGA CTG ACC GTC TGG GGA ACT AAA AAC CTC CAG
Val Val Lys Arg Gln Gln Glu Met Leu Arg Leu Thr Val Trp Gly Thr Lys Asn Leu Gln

          610         620         630         640         650         660
           *           *           *           *           *           *
GCA AGA GTC ACT GCT ATT GAG AAG TAC CTA GCA GAC CAG GCG CGA CTA AAT TCA TGG GGA
Ala Arg Val Thr Ala Ile Glu Lys Tyr Leu Ala Asp Gln Ala Arg Leu Asn Ser Trp Gly

          670         680         690         700         710         720
           *           *           *           *           *           *
TGT GCG TTT AGA CAA GTC TGC CAC ACT ACT GTA CCA TGG GTA AAT GAC ACC TTA ACA CCT
Cys Ala Phe Arg Gln Val Cys His Thr Thr Val Pro Trp Val Asn Asp Thr Leu Thr Pro

          730         740         750         760         770         780
           *           *           *           *           *           *
GAG TGG AAC AAC ATG ACA TGG CAA GAA TGG GAA CAC AAA ATC CGC TTC CTA GAG GCA AAT
Glu Trp Asn Asn Met Thr Trp Gln Glu Trp Glu His Lys Ile Arg Phe Leu Glu Ala Asn

          790         800         810         820         830         840
           *           *           *           *           *           *
ATC AGT GAG AGT TTA GAA CAG GCA CAA ATC CAG CAA GAA AAG AAT ATG TAT GAG CTG CAA
Ile Ser Glu Ser Leu Glu Gln Ala Gln Ile Gln Gln Glu Lys Asn Met Tyr Glu Leu Gln

          850         860         870         880         890         900
           *           *           *           *           *           *
AAG CTA AAT AGC TGG GAT GTT TTT GGC AAT TGG TTT GAC TTA ACC TCC TGG ATC TTC AGA
Lys Leu Asn Ser Trp Asp Val Phe Gly Asn Trp Phe Asp Leu Thr Ser Trp Ile Phe Arg
```

FIG. 21 (cont.)

```
          910         920         930         940         950         960
           *           *           *           *           *           *
CCT GGA GGA GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA TAT AAA TAT AAA GTA GTA
Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val

          970         980         990        1000        1010        1020
           *           *           *           *           *           *
AAA ATT GAA CCA TTA GGA GTA GCA CCC ACC AAG GCA AAG AGA AGA GTG GTG CAG AGA GAA
Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu

         1030        1040        1050        1060        1070        1090
           *           *           *           *           *           *
AAA AGA GCA GTG GGA ATA GGA CAG GCC AGA CAA TTA TTG TCT GGT ATA GTG CAG CAG CAG
Lys Arg Ala Val Gly Ile Gly Gln Ala Arg Gln Leu Leu Ser Gly Ile Val Gln Gln Gln

         1090        1100        1110        1120        1130        1140
           *           *           *           *           *           *
AAC AAT TTG CTG AGG GCT ATT GAG GGC CAA CAG CAT CTG TTG CAA CTC ACA GTC TGG GGC
Asn Asn Leu Leu Arg Ala Ile Glu Gly Gln Gln His Leu Leu Gln Leu Thr Val Trp Gly

         1150        1160        1170        1180        1190        1200
           *           *           *           *           *           *
ATC AAG CAG CTC CAG GCA AGA ATC CTG GCT GTG GAA AGA TAC CTA AAG GAT CAA CAG CTC
Ile Lys Gln Leu Gln Ala Arg Ile Leu Ala Val Glu Arg Tyr Leu Lys Asp Gln Gln Leu

         1210        1220        1230        1240        1250        1260
           *           *           *           *           *           *
CTG GGG ATT TGG GGT TGC TCT GGA AAA CTC ATT TGC ACC ACT GCT GTG CCT TGG AAT GCT
Leu Gly Ile Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala

         1270        1280        1290        1300        1310        1320
           *           *           *           *           *           *
AGT TGG AGT AAT AAA TCT CTG GAA CAG ATT TGG AAT AAC ATG ACC TGG ATG GAG TGG GAC
Ser Trp Ser Asn Lys Ser Leu Glu Gln Ile Trp Asn Asn Met Thr Trp Met Glu Trp Asp

         1330        1340        1350        1360        1370        1380
           *           *           *           *           *           *
AGA GAA ATT AAC AAT TAC ACA AGC TTA ATA CAC TCC TTA ATT GAA GAA TCG CAA AAC CAG
Arg Glu Ile Asn Asn Tyr Thr Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn Gln
```

FIG. 21 (cont.)

```
          1390        1400        1410        1420        1430        1440
            *           *           *           *           *           *
CAA GAA AAG AAT GAA CAA GAA TTA TTG GAA TTA GAT AAA TGG GCA CGG ATC GAA GAT CTT
Gln Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Arg Ile Glu Asp Leu

          1450        1460        1470        1480        1490        1500
            *           *           *           *           *           *
CGA TCC CGC TCC CGC CGA GCG GCT GGC GGG ATT ACC GGC TCC ATG TCC CTC GCC TCA GGA
Arg Ser Arg Ser Arg Arg Ala Ala Gly Gly Ile Thr Gly Ser Met Ser Leu Ala Ser Gly

          1510        1520        1530        1540        1550        1560
            *           *           *           *           *           *
AAG AGC CTC CTA CAT GAG GTG GAC AAA GAT ATT TCC CAG TTA ACT CAA GCA ATA GTC AAA
Lys Ser Leu Leu His Glu Val Asp Lys Asp Ile Ser Gln Leu Thr Gln Ala Ile Val Lys

          1570        1580        1590        1600        1610        1620
            *           *           *           *           *           *
AAC CAC AAA AAT CTA CTC AAA ATT GCG CAG TAT GCT GCC CAG AAC AGA CGA GGC CTT GAT
Asn His Lys Asn Leu Leu Lys Ile Ala Gln Tyr Ala Ala Gln Asn Arg Arg Gly Leu Asp

          1630        1640        1650        1660        1670        1680
            *           *           *           *           *           *
CTC CTG TTC TGG GAG CAA GGA GGA TTA TGC AAA GCA TTA CAA GAA CAG TGC CGT TTT CCG
Leu Leu Phe Trp Glu Gln Gly Gly Leu Cys Lys Ala Leu Gln Glu Gln Cys Arg Phe Pro

          1690        1700        1710        1720        1730        1740
            *           *           *           *           *           *
AAT ATT ACC AAT TCC CAT GTC CCA ATA CTA CAA GAA AGA CCC CCC CTT GAG AAT CGA GTC
Asn Ile Thr Asn Ser His Val Pro Ile Leu Gln Glu Arg Pro Pro Leu Glu Asn Arg Val

          1750        1760        1770        1780        1790
            *           *           *           *           *
CTG ACT GGC TGG GGC CTT AAC TGG GAC CTT GGC CTC TCA CAG TGG GCT CGA TCC TAG
Leu Thr Gly Trp Gly Leu Asn Trp Asp Leu Gly Leu Ser Gln Trp Ala Arg Ser ---
```

FIG. 21 (cont.)

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/06621

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): G01N 33/569; C07K 15/04, 15/14; C12N 15/00
U.S. CL.: 435/5, 320.1, 974, 975; 530/350, 395

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435/5, 320.1, 974, 975; 530/350, 395

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X Y	Nature, Volume 324, issued 18/25 December 1986, CLAVEL ET AL. "Molecular cloning and polymorphism of the human immune deficiency virus type 2" pages 691-695. see page 693.	1-2 3-12,17-24
Y	US, A, 4,753,873 (BELTZ ET AL.) 28 June 1988, see figures 19 and 21.	3-9, 13-24
Y	Proceedings of the National Academy of Sciences, USA, Vol. 80, issued June 1983, SEIKI ET AL., "Human adult T-cell leukemia Virus: Complete Nucleotide sequence of the provirus genome integrated in leukemia cell DNA", pages 3618-22. see page 3622.	7-21
Y,P	US, A, 4,939,094 (KUGA ET AL.) 03 July 1990, see column 2, lines 14-18.	3-24

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
18 March 1991	16 APR 1991
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA /US	Christine M. Nucker ebw

Form PCT/ISA/210 (second sheet) (May 1986)